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CONTENTS

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By JOSEPH M. GUILFOYLE

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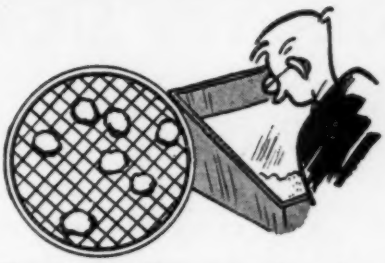

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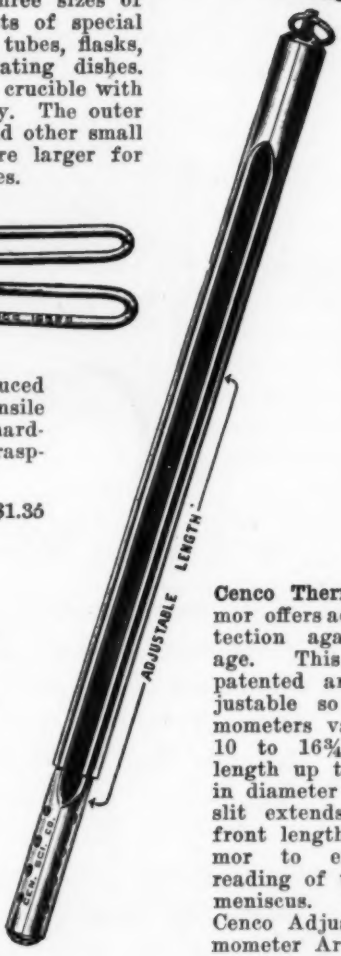

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CEREAL CHEMISTRY

VOL. XXIV MARCH, 1947 No. 2

THE RELATION OF ALPHA-AMYLASE AND SUSCEPTIBLE STARCH TO DIASTATIC ACTIVITY

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(Received for publication April 29, 1946)

Many workers have interested themselves in the relationship existing between the amylolytic enzymes of wheat and their natural substrate, raw starch. Likewise, the value of the diastatic activity figure as an indication of the potential gassing power of a flour has been the subject of much investigation and discussion. The early workers recognized that "baking strength," generally measured in terms of loaf volume, depends largely on continued sugar formation in the dough and is therefore related to the diastatic activity.

The theoretical aspects of the subject were examined by Landis and Frey (1933, 1936), who concluded that no single diastatic or saccharogenic value will give more than an approximate picture of the fermentation potentialities of a flour. They concluded that since saccharogenesis is a logarithmic function, while fermentation is essentially a linear function of time, diastasis frequently becomes the limiting factor in normal fermentation.

As early as 1879 Brown and Heron, and in 1904 Maquenne, recognized that injury to starch granules resulted in greater rapidity of diastatic attack. Alsberg (1927) confirmed this and postulated two other factors influencing fermentation rate, namely, the relative number of uninjured granules and their susceptibility. In 1924 Rask and Alsberg, in reporting a viscosimetric study of wheat starches, recalled Whymper's (1909) observations that wheat starches vary in their resistance to diastatic action according to the quantity of gluten, and they expressed doubt as to whether the diastatic figure for a flour is necessarily an expression of enzymic content alone. Collatz (1922), as quoted by Rask and Alsberg (1924), found that the starch of strong flours appeared to be more easily hydrolyzed by diastatic enzymes than that of weak flours.

Hermano and Rask (1926) also stated that starches, either raw or gelatinized, vary in susceptibility to amylases, there being no connection between the relative susceptibility in the two conditions. Landis and Frey (1933), in discussing some terms used in cereal chemistry, outlined the two factors operating in autolytic saccharogenesis, enzyme activity, and starch susceptibility. After one hour, when the small amount of highly susceptible starch has been used up, the susceptibility of the native starch is the determining factor.

Andrews and Bailey (1934) stated that the diastatic activity in normal sound wheat is due primarily to beta-amylase and they concluded that diastatic activity is more a function of the starchy substrate than of amylase activity. Read and Haas (1936) expressed the opinion that the physical condition of the starch granules influences the quantity of maltose formed.

Sandstedt, Blish, Mecham, and Bode (1937) noted that the susceptibility of starch to amylase varies in different flours but that the raw uninjured starch in these flours has the same susceptibility to a given amylase preparation. Jones (1940) studied the production of mechanically damaged starch in milling and found that at least a portion of the differences in diastatic activity between flours is due to differences in the physical hardness of the endosperm; those flours from a hard wheat have, in general, a higher maltose figure than those from a soft wheat.

The "diastatic activity" or "maltose figure," expressing the relationship between the amylolytic enzymes and their starch substrate, has been and is widely used by control laboratories as an indication of the potential gas-forming capacity of a flour. The maltose as reported originates from several different sources. There is a certain small residual amount in the flour but the greater part is formed by the action of the amylolytic enzymes during the autolytic digestion. These enzymes, two or more in number, act on a starchy substrate which may be divided on a chemical basis into two fractions, i.e., amylose and amylopectin. On a physical basis the starch is made up of variable quantities of at least three fractions. There is, therefore, the possibility of considerable variation in the amount of maltose formed.

Some of the raw or native starch granules appear visually, with the aid of iodine and congo red staining, to be intact and others to be damaged by the milling process, while still another group is apparently undamaged but stains red as do the damaged granules. Evidence indicates that the number of granules capable of being stained red is related to the amount of maltose formed (Pulkki, 1938; Dadswell and Wragge, 1940). If a more satisfactory method were available for

measuring this portion of the starch, greater progress could be made in understanding the part it plays in maltose production. In this paper it is proposed to outline such a method and to demonstrate its use in studying the variations in maltose production in flour.

Important as the substrate is in determining the magnitude of maltose production, the amount of enzyme is likewise very important and an interpretation of maltose formation is incomplete without noting the effect of variation in the amount and type of amylase.

To accomplish the objects outlined above, quantities of beta- and alpha-amylases prepared from wheat were needed, as well as amylase-free flour. In addition to the usual diastatic activity value of the original flour, an indication of the amounts of alpha-amylase, of susceptible starch, and of maltose originally present in the flours was required. Such information could then be subjected to a statistical analysis in an effort to interpret the experimental findings.

Materials and Methods

Materials. The flours studied were milled from six varieties of Australian white winter wheat grown in 1941 by the Department of Agriculture of Victoria at three places. The six varieties used, Regalia, Quadrat, Baldmin, Dundee, Ghurka, and Pindar, were chosen because they represent a wide range in maltose and gas production.

Preparation of Amylase-free Flour. Five grams of flour mixed with sand and suspended in 25 ml of 0.075 *N* hydrochloric acid at 20°–22°C were mixed by rotating the flask every 15 minutes. Forty-five minutes after the addition of acid, 1.88 ml of 1 *N* sodium carbonate were added, followed by 7.62 ml of water. Finally, within two minutes of adding the sodium carbonate and water, 11.5 ml of buffer solution, the concentration of which is four times that recommended in the Blish and Sandstedt method (*Cereal Laboratory Methods*, 3rd ed., 1935), were added at 30°C and digestion was carried out for one hour at this temperature, shaking every 15 minutes as in the usual autolytic digestion. Reducing sugars were then determined. Complete destruction of the amylases was shown by determining reducing sugars before and after digestion at 30°C.

In adding either beta- or alpha-amylase to the amylase-free flour the above procedure was followed except that only 4.62 ml of water (instead of 7.62 ml) were added, followed by 3 ml of water containing the quantity of enzyme which was desired. When enzyme was added, a correction had to be applied for its reducing value and all values obtained were corrected for the maltose originally present in the flour.

Preparation of Enzymes. The methods of extraction and precipita-

tion outlined below are based on those described by Sherman, Caldwell, and Doebbeling (1934) and later employed by Blish, Sandstedt, and Mecham (1937), and Kneen, Sandstedt, and Hollenbeck (1943). The differential inactivation of the two enzymes was carried out according to the technique first developed for malt amylases by Ohlsson (1930) and applied to those of wheat by Creighton and Naylor (1933).

Beta-Amylase. Ungerminated wheat of average quality was ground in a laboratory mill. Portions of 160 g were treated with 600 ml of 5% sodium chloride solution for 45 minutes with frequent stirring. The suspension was centrifuged and the insoluble residue extracted with a small amount of sodium chloride solution. The total volume of extract obtained was 660–670 ml.

Thirty-five grams of solid ammonium sulfate were dissolved in each 100 ml of clear extract. The resulting precipitate was separated by filtering, placed in a cellophane bag, and dialyzed for 2–3 days against running tap water with a low calcium content until free from sulfate. The contents of the bag were evaporated to dryness in a current of air at room temperature.

To destroy any alpha-amylase, each 10 g of the dry powdered enzyme preparation was mixed with 500 ml of water, cooled to 4°C, and enough 1 *N* hydrochloric acid added to bring it to pH 3.3. After 30 minutes at this low temperature the suspension was restored to pH 6 with 1 *N* sodium carbonate. The insoluble material was removed by filtration and the clear yellowish-brown solution evaporated to dryness in cellophane bags as before. The residue was finally dried in a vacuum at room temperature, finely ground, and sieved to give a uniform powder. The keeping quality of the product was excellent and it was judged to be free of alpha-amylase, since increasing concentrations, when added to a standard enzyme-free flour substrate, did not bring about any increase in dextrin formation.

Alpha-Amylase. Wheat of the same type as was used in the preparation of beta-amylase was germinated to a sprout length (plumule) of about a quarter of an inch. The germinated wheat was dried at room temperature in a current of air and ground in a laboratory mill. Extraction of the enzyme, precipitation with ammonium sulfate, and subsequent dialysis were carried out as for beta-amylase.

After addition of calcium acetate, according to the procedure suggested by Kneen, Sandstedt, and Hollenbeck (1943), in such an amount that 10 mg were present for each 5 ml of the contents of the dialyzing bags, the sulfate-free suspensions were maintained at a temperature of 75°C for 15 minutes to destroy the beta-amylase present. The suspension was then dialyzed to eliminate the calcium acetate, evaporated to dryness, and ground. The insoluble material

was not removed from this preparation before drying because the activity was of such a high order that its manipulation would not have been facilitated by further concentration.

Evidence that this alpha-amylase preparation was free from beta-amylase was obtained by use of a time-temperature inactivation curve of the type investigated by Blom, Bak, and Braae (1937), but using an amylase-free flour as substrate. A straight line was obtained, with a gradual slope, which indicated the absence of any beta-amylase.

Further confirmation of freedom from beta-amylase was obtained by examination of a curve relating the dextrinogenic and saccharogenic activity of the alpha-amylase preparation on raw starch after heating for varying periods. The regular shape of the curve indicated that beta-amylase was absent, since if it had been present there would have been initially a large decrease in maltose formation which was out of proportion to the decrease in dextrin production.

Kneen (1944, 1945) has reported that only small amounts of beta-amylase are present in a sorghum malt extract and it was found that the two methods described above were sufficiently sensitive to detect this.

Analytical Methods Used. The experimental results corresponding to the variables enumerated below are given in Table I. For each variable a symbol has been indicated, and this has been used in the tables, in the figures, and to some extent in the text.

Maltose Originally Present (*R*). Estimation of the maltose originally present was carried out using the same reagents as were needed for the determination of diastatic activity according to the Blish and Sandstedt method (*Cereal Laboratory Methods*, 3rd ed., 1935). To avoid any amylase action, it was found necessary to add the buffer and sulfuric acid previously mixed together, followed immediately by the sodium tungstate.

Susceptible Starch. The term "susceptible starch" has been used in this paper to denote that portion of the starch granules which may be estimated directly by staining, or indirectly by the use of beta- or alpha-amylases. The methods were:

(1) The staining technique carried out by Dadswell and Wragge (1940) (S).

(2) The use of beta-amylase acting on amylase-free flour (M_β. This is termed the "maltose formed by excess beta-amylase" for purposes of this discussion and may be defined as the maximum amount of maltose, in milligrams, which can be formed by allowing an excess of beta-amylase to act on 10 g of amylase-free flour in a buffered solution at 30°C for one hour. Details of manipulation are given under "preparation of amylase-free flour."

(3) The action of alpha-amylase on amylase-free flour (M_α). This is termed the "maltose formed by alpha-amylase" and it may be defined as the amount of reducing sugar, expressed as milligrams of maltose, produced by 160 mg of crude alpha-amylase when allowed to act on 10 g of amylase-free flour in a buffered solution at 30°C for one hour.

Combined Action of Amylases and Susceptible Starch. (1) Gross diastatic activity figure is the term used to include all the maltose formed during an autolytic digestion of flour and is considered to be derived from at least three sources. The main source is that supplied by the action of beta-amylase on the damaged or susceptible starch and this is influenced mainly by the amount of susceptible starch. The maltose formed thus is probably identical with the value designated above as maltose formed by excess beta-amylase and termed M_β.

The second source is that supplied by the action of alpha-amylase on susceptible starch, its magnitude being dependent on the amounts of alpha-amylase and susceptible starch.

A third and minor source of maltose derives from the action of beta-amylase on any material rendered susceptible to it by previous alpha-amylase action. If there are other amylases present they would contribute a fourth source of maltose.

By means of an amylolytic digestion carried out in steps, a better understanding was obtained of the relationship existing between alpha- and beta-amylase and between each amylase and the substrate. Amylase-free raw wheat starch was digested with an excess of beta-amylase at 30°C and washed free of soluble sugars. This material was subjected to a series of amylolytic digestions by allowing successive small equal amounts of either beta- or alpha-amylase to act at 30°C in buffered solutions. The amylase was destroyed and all soluble sugars were removed after each digestion. When the alpha-amylase digestions were interspersed with beta-amylase treatments, it was found that the amount of maltose due to alpha-amylase action was not significantly different from that obtained when no beta-amylase was used, but the amount of maltose due to beta-amylase action was increased as a result of a previous digestion with alpha-amylase, although successive treatments with beta-amylase alone gave practically no maltose. On the basis of this starch degradation, the second source of maltose in the gross diastatic activity figure is independent of the presence of beta-amylase and the third source, although due to beta-amylase, is dependent on some alpha-amylase activity having previously taken place.

For natural flours the gross diastatic activity figure (A_g) may be defined as the reducing sugar, expressed as milligrams of maltose per

10 g of flour, which has been *formed during digestion* at 30°C for one hour under the conditions specified by the Blish and Sandstedt method (*Cereal Laboratory Methods*, 3rd ed., 1935). This value differs from the ordinary diastatic activity figure in that it is corrected for the reducing sugars present originally in the flour.

For artificial flours the gross diastatic activity figure (A'_g) may be defined as for a natural flour, except that preparations of beta- and alpha-amylase were substituted in amylase-free flours for the amylases occurring in the natural flours. In these experiments 120 mg of crude beta-amylase and 140 mg of crude alpha-amylase per 10 g of flour were used.

(2) Net diastatic activity figure is the term used to designate that portion of the maltose which is formed during the gross diastatic activity estimation, and which owes its origin either directly or indirectly to the presence of amylases other than beta-amylase. It includes the second, third, and fourth sources of maltose, as noted above under the sources of maltose in the gross diastatic activity figure.

For natural flours the net diastatic activity (A_n) may be defined as the difference between the gross diastatic activity (A_g) and the maltose formed by excess beta-amylase (M_β), and is expressed as milligrams of maltose per 10 g of flour.

For artificial flours the net diastatic activity figure (A'_n) may be defined as the difference between A'_g and M_β, and is expressed as milligrams of maltose per 10 g of flour.

Alpha-Amylase (*D*). The alpha-amylase content of the flours was estimated by determination of the dextrin figure as carried out by the method of Kent-Jones and Amos (1940).

Results and Discussion

The analytical data are presented in Table I. The samples are arranged in decreasing order with respect to their susceptible starch values as based on maltose formed by excess beta-amylase.

Variety and Place Effects. The effects of variety and place in relation to the combined action of susceptible starch and alpha-amylase, as well as in relation to each factor individually, are shown by the analysis of variance in Table II. The three measures of starch susceptibility (S, M_β, M_α) indicate that variety exerts a significant effect since the variety means differ significantly among themselves. Locality means, however, differ significantly only in the cases where susceptibility of the starch is measured as maltose formed by beta- or alpha-amylase. The limitations of the method of direct measurement of starch susceptibility (S) may account for the fact that this does not show a similar relation to the variation between means of places.

TABLE I

REDUCING SUGAR CONTENT, STARCH SUSCEPTIBILITY, DIASTATIC ACTIVITY, AND DEXTRIN VALUES FOR THE DIFFERENT WHEAT FLOURS

Variety	Place	Sample number	Reducing sugars in original flours as maltose	Susceptibility of substrate as measured by			Combined effect of susceptibility of substrate, alpha- and beta-amylase expressed as maltose				Amount of alpha-amylase in original flour as shown by dextrin figure
				Starch granules 20μ diameter or over which stain with iodine–congo red	Maltose by excess beta-amylase [1]	Maltose by 160 mg crude alpha-amylase [1]	Gross diastatic activity [2]		Net diastatic activity		
							For natural flours	For artificial flours	For natural flours	For artificial flours	
			R	S	$M\beta$	M_α	A_g	A'_g	A_n	A'_n	D
			mg	%	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	%
Baldmin	Dookie	1	30	19.4	141	219	188	558	47	417	4.5
Dundee	Dookie	2	34	13.2	123	224	276	608	153	485	12.5
Baldmin	Longerenong	3	36	16.2	104	224	178	543	74	439	6.0
Dundee	Longerenong	4	44	15.2	103	188	182	532	79	429	4.5
Baldmin	Walpeup	5	26	18.5	96	196	166	503	70	407	5.0
Dundee	Walpeup	6	34	17.0	76	157	141	417	65	341	4.5
Regalia	Dookie	7	38	10.2	67	150	135	359	68	292	6.5
Quadrat	Dookie	8	23	8.0	67	150	92	315	25	248	4.0
Ghurka	Dookie	9	26	9.4	66	139	105	343	39	277	5.0
Regalia	Longerenong	10	38	12.8	61	164	143	342	82	281	8.5
Pindar	Longerenong	11	43	11.3	60	122	104	329	44	269	5.5
Regalia	Walpeup	12	30	12.8	57	134	102	333	45	276	6.0
Ghurka	Longerenong	13	47	8.8	57	134	103	359	46	302	5.0
Pindar	Dookie	14	30	8.5	56	144	92	296	36	240	5.0
Quadrat	Longerenong	15	40	10.4	51	143	94	337	43	286	5.0
Ghurka	Walpeup	16	26	8.4	43	112	84	276	41	233	5.0
Quadrat	Walpeup	17	24	8.9	36	105	68	249	32	213	5.0
Pindar	Walpeup	18	28	7.4	36	102	69	242	33	206	5.0

[1] Determinations made on amylase-free flour.

[2] Corrected for reducing sugars (R) present in original flour.

The gross diastatic activity figures of both natural and artificial flours (A_g, A'_g) are related to variety and place. This is to be expected since one of the measures of starch susceptibility (M_β) is considered to be a part of the gross diastatic activity figure. Consideration of the net diastatic activity (A_n, A'_n), where the variation in alpha-amylase should be more apparent, shows that variety means differ significantly for both natural and artificial flours, but only artificial flours containing uniform amounts of alpha-amylase show a significant variation between means of places. The same effect is somewhat apparent in the variations between means of places for the gross diastatic activity, since the significance of the difference between means is of a higher order for the artificial flours than for the natural flours.

No significant difference exists between means of varieties or places for alpha-amylase (D) and, therefore, the amount of alpha-amylase is not a feature of locality or of variety.

Starch Susceptibility. The least satisfactory of the three methods used for measurement of starch susceptibility is that involving the staining technique. Results obtained by this method were found to vary slightly with different individuals. The data enumerated in Table I were obtained by one individual. In any case it is not a method which lends itself to a high degree of accuracy and it is very tedious. The results obtained are, however, of interest to use in comparison with the data obtained by other methods.

In employing amylases to investigate the susceptibility of starch, they may be used singly or in combination. In this investigation they were used singly, since a combination simulates the more complex system of a natural flour and the results obtained would, therefore, be comparable to what has been defined above as the gross diastatic activity.

It is generally conceded that all wheat flours contain an abundance of beta-amylase and varying amounts of alpha-amylase, with the possibility of an additional raw starch-splitting amylase. In the autolytic digestion of a flour, the beta-amylase has opportunity to attack any starch fraction susceptible to it as well as dextrins formed by the alpha-amylase present. Many of the flours studied show a low alpha-amylase content and in such a case the main activity of the beta-amylase is to attack the starch which is susceptible to its action. This can be investigated by rendering a flour amylase-free, adding beta-amylase and noting the maltose formed during digestion for one hour at 30°C. When sufficient beta-amylase was added to any of the 18 samples of flour, a limiting amount of maltose was obtained which could not be increased by longer digestion or by the addition of more of the enzyme. In actual practice the value obtained must, of course,

TABLE II

ANALYSIS OF VARIANCE FOR THE EFFECT OF WHEAT VARIETY AND LOCATION OF GROWTH ON REDUCING SUGAR CONTENT, VARIOUS MEASURES OF STARCH SUSCEPTIBILITY AND DIASTATIC ACTIVITY, AND ON α-AMYLASE CONTENT—MEAN SQUARES

Source of variation	Degrees of freedom	Susceptibility of substrate as measured by			Combined effect of susceptibility of substrate, alpha-, and beta-amylase expressed as maltose				Amount of alpha-amylase in original flour as shown by dextrin figure
		Starch granules 20μ diameter or over which stain with iodine–congo red	Maltose by excess beta-amylase [1]	Maltose by 160 mg crude alpha-amylase [1]	Gross diastatic activity		Net diastatic activity		
					For natural flours	For artificial flours	For natural flours	For artificial flours	
		S	M_β	M_α	A_g	A'_g	A_n	A'_n	D
Between means of varieties	5	43.83**	2,294.62**	4,108.7**	7,174.4**	37,107**	1,818.62**	21,073	3.58
Between means of places	2	1.59	1,291.55**	2,210.1**	2,886.0*	10,837**	373.55	5,311**	2.05
Remainder	10	2.55	81.29	119.3	631.6	1,071	510.29	768	4.41

[1] Determinations made on amylase-free flour.
* Significant.
** Highly significant.

be corrected for the maltose originally present in the flour so as to obtain the amount formed by the beta-amylase. The values for these samples ranged from 36 to 141 mg of maltose per 10 g of flour.

Fair agreement between the beta-amylase method and the staining technique for the measurement of susceptible starch is indicated by the over-all correlation of 0.798 which is highly significant (see Table III). A further series of 12 samples composed of three varieties grown at four places in 1939 gave an over-all correlation of 0.763 for the same pair of factors.[1] The relationship of these two estimates of susceptible starch is such that, for a 10 mg increase in maltose, the percentage of starch granules of 20μ diameter or over which stain red increases by 1.03%.

TABLE III

CORRELATION COEFFICIENTS BETWEEN DIFFERENT MEASURES OF STARCH SUSCEPTIBILITY, DIASTATIC ACTIVITY AND DEXTRIN FIGURE

Variables correlated		Correlation coefficient
Maltose by excess β-amylase and gross diastatic activity by crude α-amylase	$r_{M_\beta M_\alpha}$	0.944**
Maltose by excess β-amylase and injured starch granules 20μ diameter or over	$r_{M_\beta S}$	0.789**
Maltose by crude α-amylase and starch granules 20μ diameter or over which stain with iodine–congo red	$r_{M_\beta S}$	0.782**
Maltose by excess β-amylase and gross diastatic activity for natural flours	$r_{M_\beta A_g}$	0.897**
Maltose by excess β-amylase and gross diastatic activity for artificial flours	$r_{M_\beta A'_g}$	0.958**
Maltose by excess β-amylase and net diastatic activity for natural flours	$r_{M_\beta A_n}$	0.609**
Maltose by excess β-amylase and net diastatic activity for artificial flours	$r_{M_\beta A'_n}$	0.926**
Maltose by excess β-amylase and dextrin figure	$r_{M_\beta D}$	0.301
Dextrin figure and gross diastatic activity for natural flours	r_{DA_g}	0.644**
Dextrin figure and net diastatic activity for natural flours	r_{DA_n}	0.853**

.16 degrees of freedom.
** Highly significant.

The third method for estimation of susceptible starch is based on the action of alpha-amylase alone on amylase-free samples of the flours studied. While this is a condition probably never present in natural flours it is of interest because of the manner in which the flours reacted. In Table I are given the responses to a uniform amount of alpha-amylase of 18 samples rendered amylase-free in the manner described in the experimental section. These values are graphically

[1] Unpublished data. Dadswell, Wragge, and Gardner, 1942.

represented in Figure 1 and show the close relationship between the responses of a given flour to the two enzymes. The quantity of maltose formed by the alpha-amylase is, of course, a function of the amount of enzyme used as well as the time of digestion, which in this case was one hour at 30°C. It may be noted here that the time-maltose relationship of each of the amylases was found to be of the nature indicated in

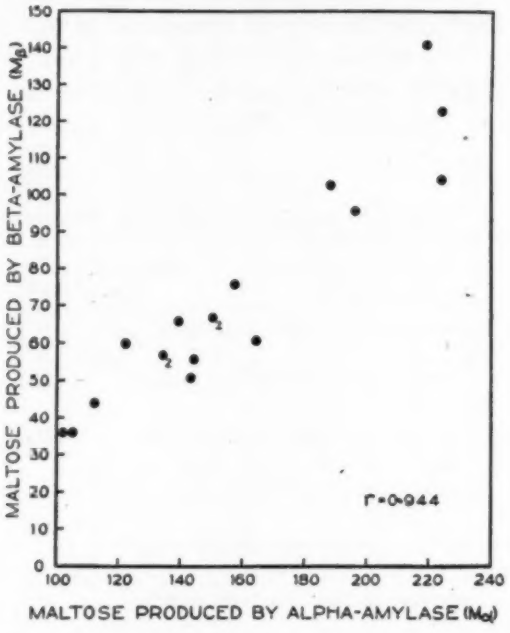

Fig. 1. Scatter diagram showing relation between maltose produced by a high level of alpha-amylase and by excess beta-amylase acting on amylase-free flours.

Figure 2. The curves illustrate the typical responses of three amylase-free flours to the two enzymes and show one of the great differences between the enzymes. The level of alpha-amylase chosen was high, so high that it is equivalent to a dextrin figure of about 30%, a value very much greater than is likely to be found in the case of a commercial flour sample. Even at this high level, the amount of alpha-amylase was insufficient to react with all the susceptible starch available in the time allowed. This constitutes one of the main differences between the two amylase methods for evaluating the susceptibility of starch.

Maltose due to alpha-amylase (M_α) is closely related to the amount of susceptible starch determined by the staining technique, as shown by their correlation coefficient of 0.782. The correlation of 0.944, indicating the relationship between maltose due to beta-amylase (M_β) and that due to alpha-amylase (M_α), is expected on account of

the close relationship of each with susceptible starch as determined by the staining method (S).

Some value such as M_α could be generally used as a measure of susceptible starch but the use of beta-amylase is more convenient and practical at present. The measurement of susceptible starch can, therefore, be most conveniently carried out by the use of beta-amylase acting on amylase-free flour.

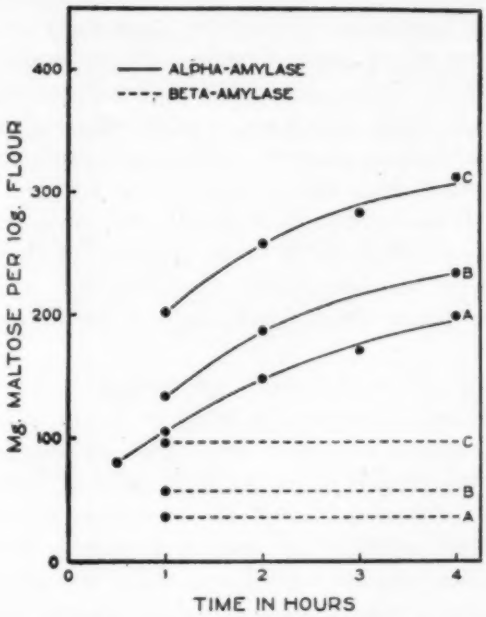

Fig. 2. Curves showing maltose production in relation to time for three amylase-free flours (A, B, and C), treated with alpha- and with beta-amylases.

Combined Action of Amylases and Susceptible Starch. The correlation between susceptible starch (M_β) and gross diastatic activity (A_g) has the highly significant value of 0.897. If starch susceptibility were measured by the staining technique (S) the correlation was also highly significant (0.694), this value being slightly reduced (0.683) if calculated on the basis of the ordinary diastatic activity, i.e., not corrected for the reducing sugars originally present in the flour. When calculated on the latter basis, a previous series of 24 samples (Dadswell and Wragge, 1940) showed a correlation of 0.954. A third series, representing four varieties grown at four places over a period of two years, gave a correlation of 0.750.[3]

For the artificial flours the correlation between susceptible starch (M_β) and gross diastatic activity (A'_g) was 0.958. The gross diastatic

[3] Unpublished data. Dadswell, Wragge, and Gardner, 1942.

activity figures for a series of artificial flours where standardized amounts of amylases were used constitute another estimate of susceptible starch; therefore, the correlation of 0.958 represents the relation between the data obtained by two methods of measuring susceptible starch, and as such its magnitude compares favorably with $r_{M_\beta M_\alpha} = 0.944$.

The values obtained for maltose due to beta-amylase (M_β) were found, in the case of each of the 18 flours, to be less than their gross diastatic activity figures (see Table I). That there is such a positive difference in favor of the gross diastatic activity figure lends support to the idea that all these flours contain one or more amylolytic enzymes, other than beta-amylase, which are capable either of forming maltose directly, or else of forming dextrins which can be split to form maltose. In one old sample of flour the gross diastatic activity figure and the maltose due to beta-amylase were nearly the same, giving values of 147 and 136 mg, a difference of 11 mg in favor of the gross diastatic activity figure. It has been noted that, as flours age, it is the net diastatic activity figure which decreases, not the maltose due to beta-amylase.

Flours having a low alpha-amylase content were found to form only small additional amounts of maltose if the original autolytic digestion period of one hour were prolonged by another hour. This indication that the beta-amylase normally present is able, within one hour at 30°C, to convert to maltose all the susceptible starch, together with the fact that addition of beta-amylase in a diastatic activity estimation does not increase the maltose value obtained, suggests that the formation of maltose during a diastatic activity determination in excess of that which beta-amylase can produce when present alone is due, directly or indirectly, to other amylases present in the original flour.

The net diastatic activity figures (A_n, A'_n) are less closely correlated with susceptible starch than are the corresponding gross diastatic activity figures, although the difference between $r_{M_\beta A'_g}$ and $r_{M_\beta A'_n}$ is not significant. The variability of alpha-amylase in the natural flours is one of the main reasons for $r_{M_\beta A'_n}$ being greater than $r_{M_\beta A_n}$. The dextrin figure was significantly correlated with the gross diastatic activity and the net diastatic activity.

The multiple correlation coefficient for the natural flours representing the relation between the factors susceptible starch, alpha-amylase, and gross diastatic activity is 0.979. The correlation between the first two factors and the net diastatic activity is 0.930 which compares favorably with $r_{M_\beta A'_n} = 0.926$, where the variability of alpha-amylase is not a factor to be considered.

Estimation of Maltose Formation. The standard deviation of the gross diastatic activity figure for the original 18 samples was 53.2 mg per 10 g of flour (Table IV). This was reduced to 24.3 mg by taking into account the susceptibility of the starch to beta-amylase, and was further diminished by consideration of the amount of alpha-amylase to give a standard error of estimate of 11.6 mg. In terms of variance this means that about 4% of the variance of the gross diastatic activity figure is still unaccounted for.

In the case of the artificial flours, the standard deviation of the gross diastatic activity figure is 113, which is reduced to a standard error of estimate of 33. This leaves about 8% of the variance still unaccounted for and compares favorably with the amount of variation unaccounted for in the natural flour.

TABLE IV

ORIGINAL AND RESIDUAL VARIANCES OF DIASTATIC ACTIVITY [1]

Original variance for	Residual variance after correction by regression on			Percentage, residual variance of original after correction by			Percentage of original variance accounted for by		
A_g 2830.2	$M\beta$ 553.0	D 1656.4	$M\beta D$ 117.6	$M\beta$ 19.5	D 58.5	$M\beta D$ 4.2	$M\beta$ 80.5	D 41.5	$M\beta D$ 95.8
A_n 882.1	554.9	240.3	119.2	62.9	27.2	13.5	37.1	72.8	86.5
A'_g 12814.2	1053.8	—	—	8.2	—	—	91.8	—	—
A'_n 7259.0	1034.6	—	—	14.2	—	—	85.8	—	—

Percentage of residual $A_n.D$ = 240.3 accounted for by $M\beta\,\beta$ 50.4, unaccounted for by β — 49.6%.
Percentage of A'_n accounted for by $M\beta\,\beta$ 85.7, unaccounted for by $M\beta\,\beta$ 14%.

[1] Key to symbols:

A_g = Gross diastatic activity for natural flours.
A'_g = Gross diastatic activity for artificial flours.
A_n = Net diastatic activity for natural flours.
A'_n = Net diastatic activity for artificial flours.
D = Amount of alpha-amylase in original flour as shown by dextrin figure.
$M\beta$ = Maltose by excess beta-amylase.

The dependence of the gross diastatic activity figure on the susceptible starch becomes less strongly marked when alpha-amylase is included as a factor, the net regression coefficient for susceptible starch being reduced from 1.610 to 1.388,[3] but this is more than compensated for by the increase in the accuracy of the estimate brought about by introducing the second factor.

The standard deviation of the net diastatic activity figure for the original flours has been lowered from 29.7 to give a standard error of estimate of 11.6 mg per 10 g of flour by inclusion of the effects of sus-

[3] See equations, Figure 3.

ceptible starch and alpha-amylase. This leaves 14% of the variance unaccounted for.

The artificial flours have a standard deviation of 85 for the net diastatic activity figure which is reduced to a standard error of estimate of 33 by consideration of the effect of susceptible starch. The variance unaccounted for is 14% which is the same as for the natural flours. Such a comparison is open to question, since in one case the variance unaccounted for is based on conditions of uniform alpha-amylase

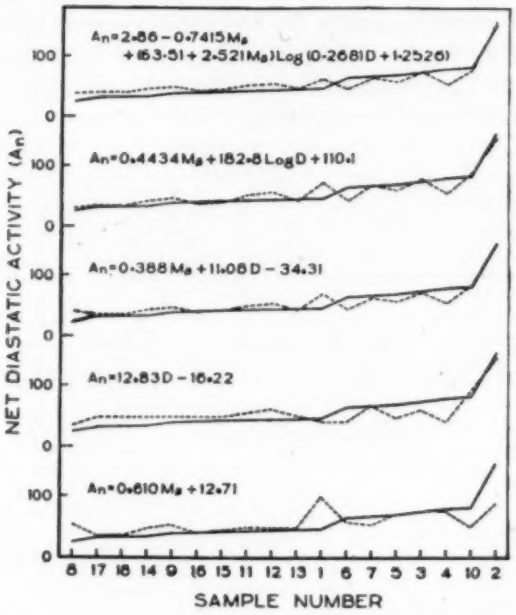

Fig. 3. Actual (————) and estimated (– – – –) values of net diastatic activity.

content, while in the other case it is based on varying alpha-amylase content. A comparison can be made, however, after the variance due to alpha-amylase has been eliminated by calculation. On that basis the variance unaccounted for by susceptible starch is 7.1% in the case of the natural flours as compared with 8.2% in the case of the artificial flours, and 50% for the net diastatic activity of the natural flours as compared with 14% for artificial flours. That the variance unaccounted for is so much higher for the natural flours than for the artificial flours indicates that either the experimental error is much higher for natural flours or there is a possibility of some unrecognized variant being present in the natural samples.

Comparisons of the experimentally determined values for the net diastatic activity figure with those calculated from the susceptible

starch or the alpha-amylase content or from both factors have been made in Figure 3. Use of both factors together gives the best estimate of the net diastatic activity, but, as would be expected from the previous discussion, susceptible starch alone gives a better estimate than alpha-amylase, except in six cases. The curve obtained from the equation $A_n = 0.4434M_\beta + 182.8$ Log.D $- 110.1$ is also shown in Figure 3, and differs little from that based on the simpler relationship between susceptible starch and alpha-amylase.

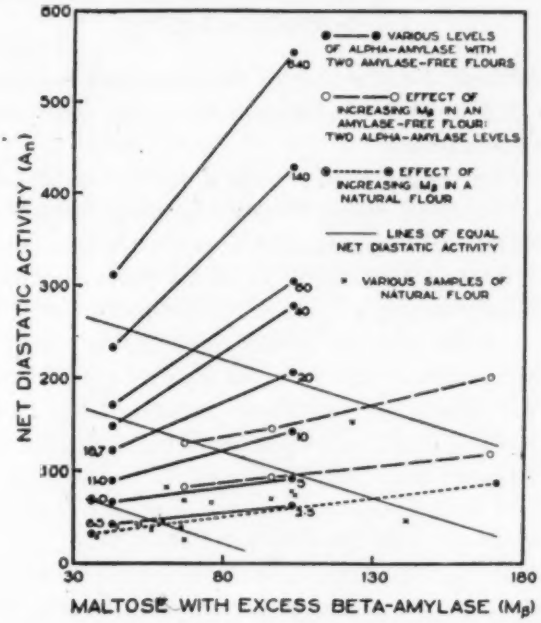

Fig. 4. The relationship between maltose produced by excess beta-amylase and net diastatic activity at various levels of alpha-amylase in the presence of excess beta-amylase. (The figures beside the curves on the right-hand side represent the units of alpha-amylase used per 10 g of flour; those on the left-hand side indicate the equivalent % dextrin.)

The 18 natural flours studied present a limited range of both alpha-amylase content and susceptible starch and, consequently, a prediction of the net diastatic activity for other combinations of the two factors is difficult. By using the data obtained from the treatment of amylase-free flour with an excess of beta-amylase in the presence of varying levels of alpha-amylase, it is possible to extend the information obtainable and so to predict the net diastatic activity of flours having a wide range of alpha-amylase and susceptible starch values.

It was observed (Figure 4) that the net diastatic activity figures of the artificial flours having a susceptible starch value of 43 are a logarithmic function of the amount of alpha-amylase used and of the percentage of dextrin, the equation being $A_n = 171.9$ Log(0.2681D

$+ 1.2526) - 29.025$. The increase in the value of the net diastatic activity over the range of susceptible starch from 43 to 103 is given by the expression $[151.24 \ \text{Log}(0.2681D + 1.2526) - 44.49] \frac{M - 43}{60}$. These two equations together give the value of the net diastatic activity figure corresponding to any value of susceptible starch between 43 and 103, and varying levels of alpha-amylase,

$$A_n = 2.860 - 0.7415M_\beta + (63.51 + 2.521M_\beta) \text{Log}(0.2681D + 1.2526).$$

The values calculated according to this equation for the 18 samples are shown in Figure 3, and compare favorably with those obtained from the other equations.

Dextrin values as given in Figure 4 for the artificial flours have been corrected so as to make them comparable with those obtained for natural flours. The relationship between the two series was found to be $D = 3.002 \times \%$ dextrin for artificial flours $- 2.65$. The cause of this discrepancy is not at present understood, but it is considered that the explanation may lie in the effect of the acid on the starch during the preparation of the artificial flour as a substrate, in a difference in the alpha-amylase in the preparation used from that as it exists in the natural flour, as it appears to be less active when isolated and allowed to react with amylase-free flour in the presence of excess beta-amylase, or in the possible presence of an activator in the natural flour which is absent from the artificial flour.

One point to be elucidated is the variable response, in terms of maltose production, of ordinary commercial flours with the same diastatic activity figure to the addition of equal amounts of alpha-amylase. Examination of Figure 4 may assist in clarifying this problem. The solid radiating lines, marked as being equivalent to certain levels of dextrin formation, were determined experimentally by subjecting amylase-free flours with different susceptible starch values to a given addition of alpha-amylase in the presence of an excess of beta-amylase. The dextrin values given have been corrected as indicated earlier so as to be comparable with those obtained for natural flours. Each line shows the effect of holding the alpha-amylase at a constant level while varying the value of susceptible starch, with its resultant effect on the net diastatic activity. Each of the three parallel lines running between the two axes represents a series of possible flours having the same gross diastatic activity figures, i.e., the values of the two axes add up to the same amount along a given line, for instance, $M_\beta = 150$ plus $A_n = 50$ equals a gross diastatic activity

of 200, or $M_\beta = 75$ plus $A_n = 125$ equals a gross diastatic activity of 200. The dot and dash lines represent the effect of varying the susceptible starch while keeping the alpha-amylase content the same. This was accomplished by grinding a flour very slightly in a ball mill and sampling it at intervals. In this case the amylases naturally present were not destroyed, nor were they augmented by any additions but were allowed to act in the usual autolytic digestion. These curves confirm the solid radiating curves which were made up by observing the behavior of amylase-free flour treated with preparations of alpha- and beta-amylases.

The effect of adding a given amount of alpha-amylase to flours with different susceptible starch values is dependent on whether M_β is large or small. If large, the enzyme is very effective in raising the net diastatic activity figure and hence the gross diastatic activity figure, but if M_β is small, an increase in alpha-amylase increases the net diastatic activity value relatively little. For instance, if $M_\beta = 125$ and $A_n = 49$, with a resultant gross diastatic activity of 174, an increase in alpha-amylase equivalent to 6% dextrin will increase A_n to 135, that is by 86 mg of maltose, resulting in a gross diastatic activity of 260. On the other hand, if $M_\beta = 50$ and $A_n = 35$, with a resultant gross diastatic activity of 85, an increase of the same magnitude in alpha-amylase will result in A_n being increased to 79, a net increase of only 44 mg of maltose with a gross diastatic activity of 129.

The variable response of commercial flours to addition of alpha-amylase is therefore capable of a simple explanation in that it depends to a considerable extent on the level of susceptible starch in the flour.

Summary

Flours prepared from six varieties of white winter wheat, each grown at three places in Victoria, Australia, were studied with reference to their susceptible starch, alpha-amylase, and diastatic activity.

A corresponding series of artificial flours, with the same susceptible starch content as the natural flours but having a uniform amylase content, was also studied with reference to diastatic activity.

Susceptible starch was found to be related to variety and place of growth, but alpha-amylase bore no relation to either. In natural flours, containing variable levels of alpha-amylase and susceptible starch, the gross and net diastatic activity figures mainly reflected the effect of variety. In artificial flours the gross and net diastatic activity figures were both significantly related to place and variety effects.

The gross and net diastatic activity figures were significantly correlated with the susceptible starch and alpha-amylase contents of the natural flours, and with the susceptible starch of the artificial flours.

In the gross diastatic activity figures of the natural flours about 80% of the total variance is accounted for by the variability of the susceptible starch and an additional 16% by the alpha-amylase, leaving only 4% of the original variance still unaccounted for.

In the gross diastatic activity figures of the artificial flours about 92% of the total variance is accounted for by the variation in susceptible starch, which is similar to that accounted for by the same factor in the natural flours (96%) when calculated on the basis of uniform alpha-amylase content.

In the net diastatic activity figures of the natural flours about 37% of the variance is accounted for by the variability of the susceptible starch and about 73% by the alpha-amylase. When the susceptible starch and alpha-amylase are used together to predict diastatic activity the total variance accounted for is about 86%.

In the net diastatic activity figures of the artificial flours about 86% of the variance is accounted for by variation in susceptible starch. For the natural flours the variance for net diastatic activity after correction for the dextrin figure is 240.27 and this is reduced to 119.17 after taking into account the effect of variation in susceptible starch. The portion of 240.27 accounted for here is 121.10 or only 50%. This discrepancy suggests that some factor other than susceptible starch and alpha-amylase may be necessary to explain the variability of the net diastatic activity.

A better understanding of the variable response to alpha-amylase, in terms of maltose production in natural flours having the same gross diastatic activity figure, has been attained by reference to their susceptible starch content; flours having a high susceptible starch content giving a greater maltose production for a given amount of alpha-amylase than those having a low amount of susceptible starch.

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A STUDY OF THE BEHAVIOR OF NONVIABLE DRY YEAST IN BREAD DOUGH

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The general utilization of dry nonviable yeast as a food presents more of a problem than does its production. Brewers' by-product yeast and primary grown yeasts have been dried and made available to the pharmaceutical trade for special dietary uses for many years. In recent years, dehydration equipment and processes have come into more common use, and food and feed shortages, along with the pressure of wartime requirements, have increased the market demand for dry yeast.[2] The brewing industry has responded to this demand by increasing its yeast recovery to a potential of approximately 5 to 10 million pounds of dry yeast yearly with from 15 to 20 drying plants operating in breweries and yeast collecting centers. This represents only a portion of the ultimate potential, variously estimated at from 20 to 30 million pounds yearly. Primary grown yeast production has been perfected to give high yields, and in areas where fermentable carbohydrates are plentiful this could be made a source of high-quality protein and vitamin-rich food material.

Dry brewers' yeast has found an outlet in animal feed mixes, and, after debittering, has been used for direct human consumption as a special dietary supplement. Many food processors have found dry yeast useful in limited amounts in supplementing the vitamin and protein content and flavor qualities of their products. A more extensive use of dry yeast as a food will require special means of preparing or incorporating the yeast because the palatability of dried yeast does not make it generally acceptable.

Yeast is a natural component of yeast-leavened bread, and it would appear that additional yeast in the nonviable form would be a logical addition for enhancing its nutritional value. Such a procedure would afford a means for the ultimate consumption of this highly nutritious material. Incorporating dry yeast in bread was experimented with in Europe during the first World War and it was concluded that only limited amounts could be used to make a more nutritious yet palatable product. Schülein (1935) found that additions of 3% dry irradiated yeast to bread definitely improved its nutritional value.

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[2] The term dry yeast as used throughout this paper refers to nonviable dry yeast unless otherwise specified.

Schwarz, Laufer, Laufer, and Brenner (1942) found that additions of 2.5% and 5.0% (flour basis) of dry brewers' yeast to white bread made significant contributions to the vitamin B-complex content of the resulting loaf. Light and Frey (1943) found that the addition of 5% dry yeast, containing approximately 50% protein, made a very definite improvement in the nutritional value of bread, as measured by animal growth response tests, in comparison with growth response in a control, and with bread from a 6% nonfat milk solids formula. The growth response was proportional to the lysine increase from the respective additions of the dry yeast and the nonfat milk solids. Because white flour protein is somewhat deficient in this essential amino acid, dry yeast, which has a good proportion of lysine in its protein, becomes a logical supplement for improving the deficiency and establishing a better nutritional balance in the white bread.

Recently, McCollum (1945) has suggested the use of dry brewers' yeast and other protein-rich and vitamin-rich natural products as sources for "natural" enrichment. He indicated that bread supplemented with these materials gave loaves of good palatability, but which did not score high by conventional scoring methods. He suggested that if such materials were used, the consumer might have to modify his ideas of what constituted good quality bread.

Schwarz and co-workers found that the loaf score decreased as the amount of dry yeast was increased, and the addition of 2½% dry yeast produced a loaf scoring slightly below normal.

It is generally known by those who have included dry yeast in the dough batch that a pronounced dough softening effect is likely to be encountered when the dough is being mixed. The dough condition may improve to some extent during fermentation, but the deterioration persists throughout the breadmaking process, resulting in inferior bread.

Subsequent to the work of Schwarz *et al.* (1942) in this laboratory, it was found that commercial dry yeasts vary considerably in the degree to which they affect the physical characteristics of the dough. It appears that the sample of dry yeast with which they experimented might have been somewhat better than the average commercial dry yeast in this respect. An investigation of this variable factor in dry yeast forms the basis of this report.

Materials and Methods

A number of dry yeast samples were obtained from widely scattered sources and subjected to comparative tests. A brief description of the yeasts is given in Table I. In some cases, the method of processing these yeasts was known, but no attempt was made to correlate the

processing treatments and drying methods with the experimental findings.

Because the deleterious effects of nonviable dry yeast in a bread dough are evident in the finished loaf, as well as in the mixing and handling of the dough, a baking test was used to evaluate the bread-making properties of the dry yeast samples.

TABLE I

BREAD SCORES WHEN THE FORMULA INCLUDES 3% (FLOUR BASIS) OF VARIOUS DRIED NONVIABLE YEASTS AS RECEIVED AND AFTER DIFFERENT TREATMENTS

Dried yeast sample		Mg iodine reduced by water extract from 1 g dried yeast (as received)	Bread score[1]		
Identification No.	Descriptive origin of yeast		Yeast used as received	Yeast used after treating with sodium chlorite, 1 mg/g yeast	Used after boiling 1 hour, then treating with sodium chlorite, 1 mg/g yeast
Control	——	—	100	—	—
L	Lager yeast, debittered	1.1	95	—	—
M	Primary grown nutritional yeast	2.4	92	—	—
I	Lager yeast, debittered	3.5	85	93	—
H	Ale yeast, debittered	4.3	84	94	—
D	Ale yeast, not debittered	4.3	84	94	—
G	Ale yeast, debittered	4.3	82	94	—
K	Ale yeast, not debittered	5.0	82	94	—
B	Lager yeast, not debittered	4.3	80	84	94
F	Primary grown nutritional yeast	5.3	80	88	94
N	Compressed bakers' yeast, laboratory dried[2]	5.3	76	88	92
O	Comm'l bakers' viable dry yeast[2]	3.7	76	88	94
J	Lager yeast, not debittered	4.7	75	84	94
A	Lager yeast, debittered	5.6	75	80	90
E	Lager yeast, debittered	5.9	75	86	93
C	Lager yeast, not debittered	5.6	50	40	92
15 mg % glutathione (GSH)			75	98[3]	

[1] A score of 100 was assigned to the control in each day's bake. Scores of 95 and over: excellent; 90–94: good; 85–89: fair, acceptable; 80–84: poor; below 80: very poor.

[2] Suspension of the dried bakers' yeast pasteurized at 60°C for 15 minutes to kill viable yeast before use.

[3] 15 mg glutathione (GSH) in solution was satisfactorily oxidized by pretreatment with 4 mg sodium chlorite.

The baking test was based on the use of 400 g bakers' patent flour mixed in a C-10 Hobart-McDuffy type mixer. Other ingredients were water (62% flour absorption, 15% moisture basis), compressed yeast (3%), salt (2%), hydrogenated shortening (3%), and sugar (5%). Dry yeast was used in amounts of 3% and 5% (flour basis) and additional water was calculated at the rate of approximately 50% of the weight of the added dry yeast. A portion of the formula water was

used to incorporate the dry yeast in a slurry before adding it to the mixer with the other ingredients. The normal mixing time for the control was three minutes at low speed and two minutes at medium speed. When dry yeast was added, the mixing time at medium speed was reduced according to the necessity imposed by the dough-softening effect of the dry yeast under test; most of the samples required a reduction in mixing time of 15 to 30%.

Doughs were fermented at 27°–29°C for 105 minutes to the first punch, 45 minutes to the second punch, and 25 minutes to the pan. From each mix, two 10-ounce doughs were panned and proofed at 35°C for approximately 55 minutes. A constant height was used as the criterion for correct proof time unless the dough did not reach normal height in 60 minutes. In this case, the dough was placed in the oven regardless of height. The loaves were baked for 35 to 40 minutes at 230°C.

Sponge dough tests were also made to confirm the reliability of the indications from the straight dough procedure. Bread scoring was based principally on the factors of loaf volume and texture, grain, and color of the crumb because these were the characteristics most directly associated with the factors under study in these experiments.

Experimental Results and Discussion

Preliminary studies with dry yeasts indicated that their inclusion in the bread formula had little effect on gas production. Also, the factor or factors in the dry yeast that caused the destructive effect on the physical character of the dough were found to be in the water-soluble extract of the dry yeast. When dry yeast made up into a slurry was centrifuged before incorporating in the mixer, and only the supernatant liquid used, the deleterious effect on the dough was equal to that of the entire yeast. When the yeast slurry sediment was washed and incorporated in the dough, only a slight harmful effect was observed.

Jørgensen (1936), Ford and Maiden (1938), and Swanson and Andrews (1945) have shown, with mixograms, a softening effect of the water extract of boiled yeast on bread dough similar to the action of glutathione in a dough. It was our experience that dry yeast, because of certain water-soluble factors, behaves in a dough similar to glutathione (GSH) or other reducing substances such as sulfites. When 15 mg glutathione per 100 g flour (15 mg percent) was included in the bread formula, the dough-handling properties and loaf score were similar to those obtained when 3% of the average dry yeast was used.

Figure 1 shows the effect on loaf characteristics of adding 3% (flour basis) of the various dried yeasts to the bread formula. Two

controls are used for reference. Loaves marked "Bl" represent the blank or control to which no dry yeast additions were made. The loaf marked "Gl" represents the loaf deterioration caused by the

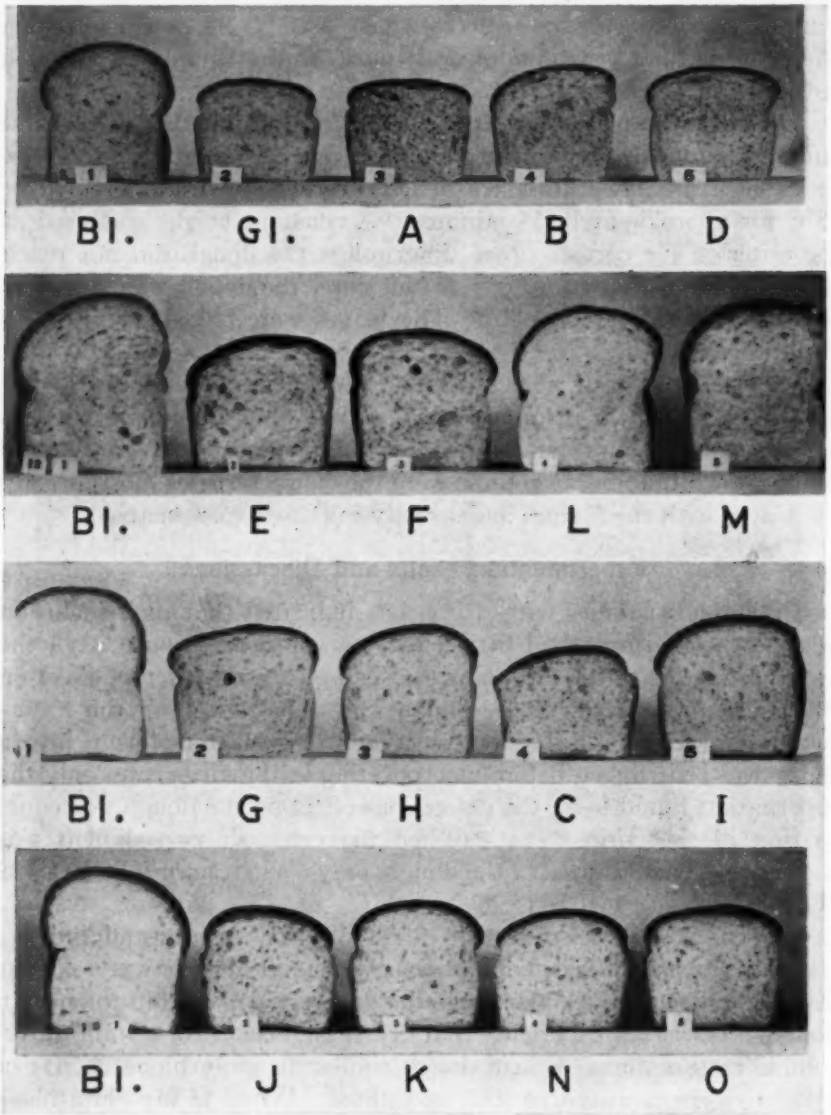

Fig. 1. Effect of adding 3% (flour basis) of various dried nonviable yeasts, untreated, to the bread formula.
Bl = blank, no dry yeast added.
Gl = glutathione added, 15 mg percent.

addition of 15 mg percent of glutathione (GSH). There is considerable variation in the effect of various dry yeasts on the bread. Only

yeasts *L* and *M* gave loaves classified as satisfactory. The next step was to investigate the possibilities of improving the breadmaking properties of the yeasts producing poor loaves.

Because the low-scoring loaves had the characteristics of bread requiring oxidizing agents, experiments were conducted along these lines. The effect of oxidizing agents in dough fermentation appears much later than the softening effect of dry yeast. For this reason, the use of oxidizing agents in the bread formula did not prevent the dough softening in the mixer; however, the softening action occurring in the mixer, in most instances, appeared to be reversible when the oxidizing agent had exerted its effect as fermentation progressed. A more direct method of eliminating the reducing substances in the dry yeast was to treat the yeast in a slurry (1 part yeast, 2 parts water) with the oxidizing agents before adding to the dough batch. Several common oxidizing agents, potassium bromate, potassium iodate, sodium chlorite, and hydrogen peroxide, were used in varying amounts with yeast *G*, which represented a yeast having an average dough-softening effect in the mixer. Hydrogen peroxide and sodium chlorite both gave rapid and effective oxidation. Conducting all treatments at room temperature (20°–25°C), the use of sodium chlorite to the extent of 1 mg per gram of dry yeast for 20 to 30 minutes before mixing gave optimum improvement for the average dry yeast. This rate of treatment is obviously not the optimum degree of oxidation for this entire lot of samples, which varied considerably in their dough-softening properties in the mixer, but this level proved to be a good working basis for improving all samples needing treatment.

All of the yeasts not giving a satisfactory score, when used at the 3% level, were treated with sodium chlorite in the manner already described. The baking results are shown in Figure 2 and Table I. This treatment gave a very definite loaf score improvement for all yeasts except *C*. Yeasts *A*, *B*, *E*, *F*, *J*, *O*, and *N* gave loaves that were acceptable, but could not be rated good. Varying the amounts of chlorite treatment did not give the desired improvement with these yeasts.

With yeast *C*, the loaf score decreased as a result of this treatment. When treated with chlorite it produced dough that became progressively softer during fermentation, similar to the effect of adding papain to a dough formula. This progressive softening was slightly more pronounced after the chlorite treatment than when used as received. The response of this sample likewise was not improved by varying the amount of the chlorite treatment. Because of the progressive softening of this dough during fermentation, and because the iodine titration

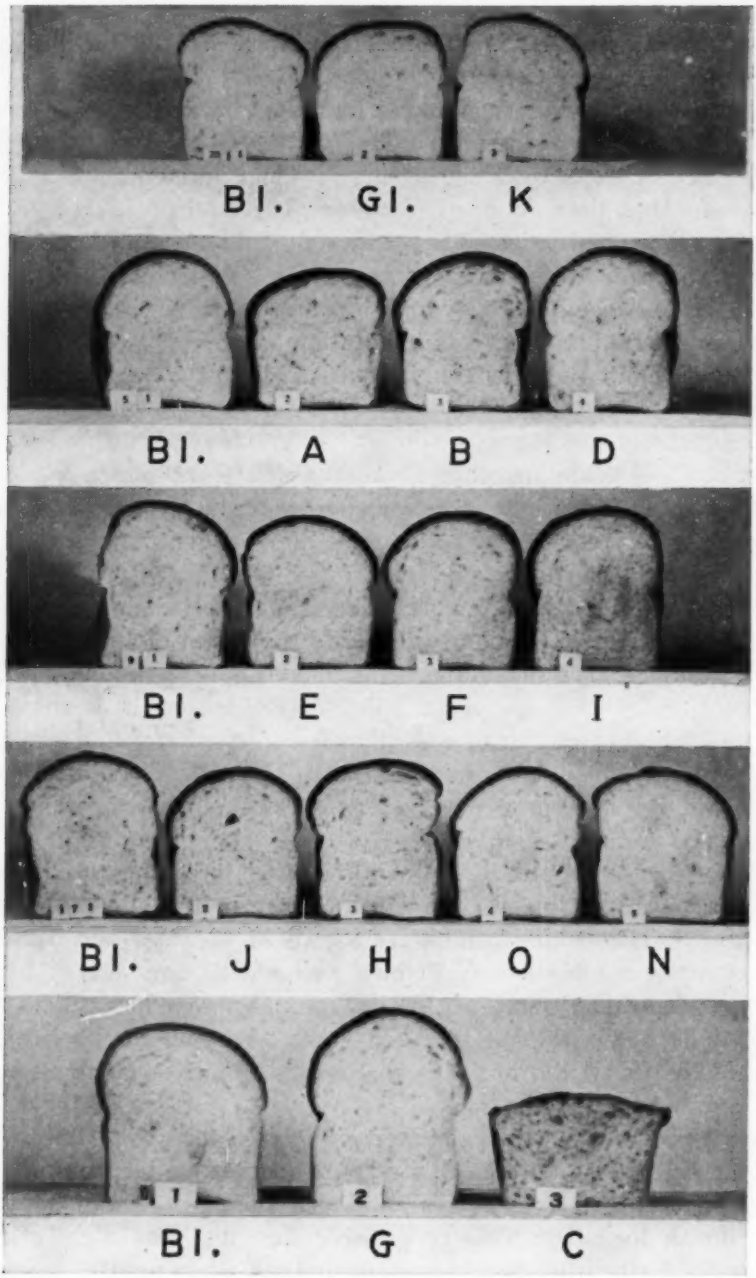

Fig. 2. Effect of adding 3% (flour basis) of poor baking nonviable dried yeasts after the yeast had been treated with sodium chlorite (1 mg per gram).
Bl = blank, no dry yeast added.
Gl = 15 mg percent oxidized with 4 mg sodium chlorite.

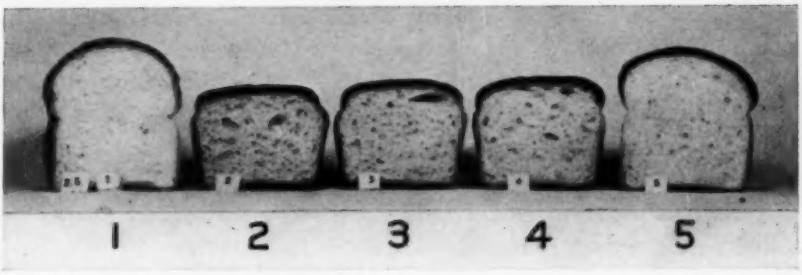

Fig. 3. Showing the development of baking improvement in a dry yeast originally giving very poor baking results.

1. Blank, no dry yeast in formula.
2. 3% dry yeast *C* added as received.
3. 3% dry yeast *C* added after treatment with sodium chlorite (1 mg per gram) 30 minutes before dough mixing.
4. 3% dry yeast *C* added after boiling 1 hour before adding to the dough mixer.
5. 3% dry yeast *C* added to the mixer after boiling 1 hour and subsequent treatment with sodium chlorite (1 mg per gram).

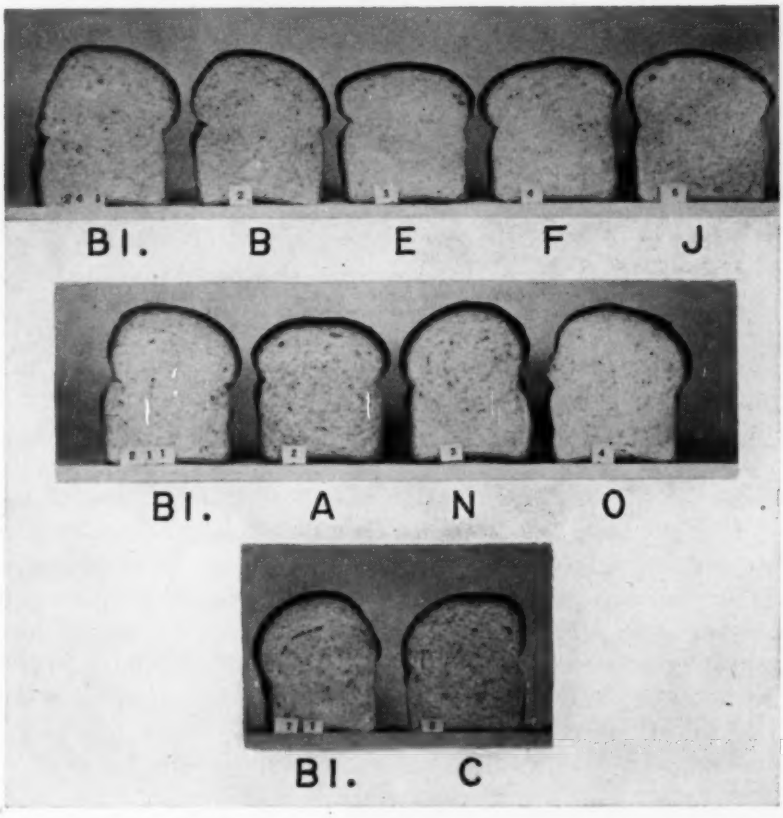

Fig. 4. Effect of treating poor baking nonviable dry yeasts with 1 hour boiling and subsequent sodium chlorite treatment (1 mg per gram) before inclusion in the bread formula. 3% dry yeast (flour basis) in formula.

Bl = blank, no dry yeast in formula.

value [3] (as a measure of reducing substances) was not increased on heating this yeast, the effect was assumed to be enzymatic in character. Heat treatment was therefore employed to correct this condition. Figure 3 shows the result of both chlorite and heat treatment used separately and in combination on yeast *C*. The combined heat and chlorite treatment was necessary to produce acceptable bread with this yeast. This combined heating and chlorite treatment was next applied to all yeasts that did not give a loaf scoring "good" by the sodium chlorite treatment only. Figure 4 and Table I show that the combined treatment gave satisfactory bread with the remainder of the dry yeast samples.

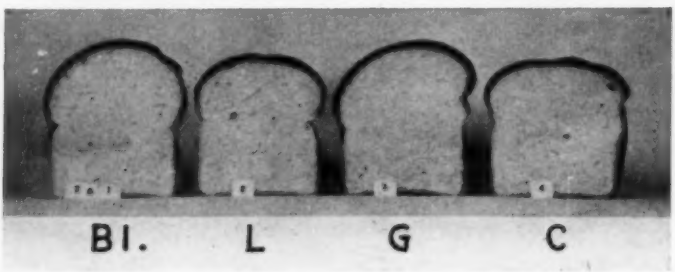

Fig. 5. Results of using 5% properly treated dry yeasts in the bread formula. These yeasts differed greatly in their original functional behavior in the dough.

Bl No dry yeast added.
L Yeast *L* with no treatment.
G Yeast *G* with sodium chlorite treatment only (1 mg per gram).
C Yeast *C* with boiling and sodium chlorite treatment.

Among the yeast samples used in these experiments three types of reactions in bread dough were encountered. Samples such as yeast *L* could be used at the 3% level as received without seriously affecting the loaf score. Others, such as yeast *G*, responded to the oxidizing (sodium chlorite) treatment to give improved baking results, while still other samples, such as yeast *C*, required heating and oxidizing treatment to produce the desired results. Yeasts representing each of these types were given their required treatment and used in bread to the extent of 5% (flour basis). The results indicated that, from the standpoint of dough-handling properties and factors related to gluten structure in the loaf, as evidenced by satisfactory loaf volume, grain, and texture (Figure 5) it is possible to use this amount of properly treated dry yeast in bread. The effects on the dough, however, were

[3] The iodine titration method used yeast equivalent to 1 g dry yeast to which was added 10 ml water (20°-25°C) in a 15 ml centrifuge tube. The suspension was agitated thoroughly for 1 minute and let stand for 10 minutes, then centrifuged. Five ml of the supernatant was diluted to about 50 ml with water, then 1 ml 1*N* H_2SO_4 and 1 ml 2% starch solution were added. This mixture was titrated directly with .01 *N* iodine solution and the titration values calculated to milligrams of iodine per gram of dry yeast.

such as to indicate that this rate of use approaches the maximum even for a properly treated dry yeast.

In experiments where living yeast cells were made nonviable by heating, freezing, or mechanical treatment, there was a pronounced increase of reducing substances as measured by an iodine titration of an acidulated water extract of the yeast. The ordinary drying of yeast also causes a great increase in the iodine titration values of the water extract of the yeast. It appears that any means used to render yeast cells nonviable releases these reducing substances, which are similar to GSH groups in their effect on bread dough. Viable yeast can be made nonviable by heating to pasteurization temperatures, and with the reducing substances then released from the yeast cells they can be oxidized with some active oxidizing agent such as sodium chlorite or hydrogen peroxide before drying. When these substances have been properly released and oxidized the yeast can then be dried without further release of such materials.

The iodine titration values show a fairly good relation with the ultimate baking results of the untreated yeast (see Table I). In Figure 1, loaves *E*, *A*, *L*, and *M*, representing the poorest and best of the yeasts, correspond to iodine titration values of 5.9 mg, 5.6 mg, 1.1 mg, and 2.4 mg. In other series of yeast samples, a preliminary selection of the good and poor baking samples could be made by using this simple test. A test of this type with the added refinement of protein precipitation might be a simple, reliable control method for appraising the bread-baking value of nonviable dry yeast, particularly since the major dough impairing factors in dry yeast appear to be of the nature of reducing substances.

The behavior of these dry yeast samples, particularly sample *C*, which was dried in a low temperature process, suggests that in some processes proteolytic enzymes may survive the drying although the yeast loses its fermenting ability and viability. By elevating the heat treatment temperature in the yeast slurry to boiling, the proteolytic enzymes can be destroyed and the reducing substances which are released can then be oxidized before drying. This affords a practical procedure for eliminating both the reducing and enzymatic factors in producing nonviable dry yeast suitable for incorporating in bread dough.

This paper has been concerned only with the functional behavior of nonviable dry yeast as an ingredient in bread doughs. It is of interest to know how the recommended treatments for making yeast useful as a bread ingredient affect its nutritional values. Two yeast samples properly treated so that 5% (flour basis) could be added to

the bread gave the following average enrichment level with respect to some of the vitamin B-complex factors:

	Thiamine mg/lb	Riboflavin mg/lb	Niacin mg/lb
Control	0.25	0.27	3.6
Bread including 5% dry yeast	2.72	.78	8.8

Although these data are not extensive, it is evident that the treatment of the yeast does not destroy its value as a vitamin supplement for bread.

Summary

Commercial nonviable dry yeasts vary considerably in the extent to which they affect bread dough when they are used in the bread formula.

Nonviable dry yeasts having a deleterious effect in bread dough can be improved by proper oxidizing treatment, or heating and oxidizing treatment, before being incorporated in the dough.

Two independent factors appear to cause nonviable dry yeast to soften bread doughs. The predominating factor behaved as though it consisted of reducing substances, and the second factor behaved as though it were enzymatic in character. In most of the dry yeasts the reducing substances were the predominant dough-softening factor, and an iodine titration of the water extract was found to be useful in predicting the baking value of the dry yeast.

These studies suggest that dry yeast of improved functional bread-baking qualities can be produced by heating the yeast to boiling temperatures before drying to release the reducing substances from the cell and to destroy proteolytic enzyme activity. The released reducing substances can then be oxidized before drying to produce a yeast of improved baking value.

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INVESTIGATION OF THE PROXIMATE CHEMICAL COMPOSITION OF THE SEPARATE BRAN LAYERS OF WHEAT [1]

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Although the proximate composition of commercial bran is well known (Bailey, 1944), little information is available on the proximate chemical constitution of the individual layers of cells which compose bran. Goncalves (1944) gives data in which the bran was divided into (1) epidermis and cross layers, (2) testa and hyaline, and (3) aleurone. No method was given for the separations. Techniques by which individual bran layers may be separated from each other have hitherto been of a mechanical nature and too tedious and time-consuming to provide sufficient material for analysis.

Preliminary work in this laboratory led to a process for separating the bran layers from each other by a combination of physical and chemical methods. The process was applied to yield sufficient material for quantitative determinations of moisture, ash, protein, fat, crude fiber, cellulose, and pentosans. The results are presented in this paper.

Materials and Methods

Four commercial blends of wheat were employed. Sample 1 was a hard red spring blend used for the production of a family flour, while sample 2 was a hard red spring blend used in producing a bakers' flour. Sample 3 was a Michigan white wheat, while sample 4 was an Ohio soft red winter wheat.

The individual bran layers will be designated as shown in Figure 1. The epidermis was removed by suspending the wheat in water and stirring in a Waring Blendor equipped with rubber beaters. It was necessary to reduce the speed by means of a variable resistance. This treatment removed the epidermis completely except in the crease. The cross layer and testa were next removed as follows The air-dried peeled wheat was soaked in a nearly saturated solution of sodium hydroxide in 95% ethyl alcohol overnight, the supernatant liquid poured off, and 95% ethyl alcohol added. The testa layer was

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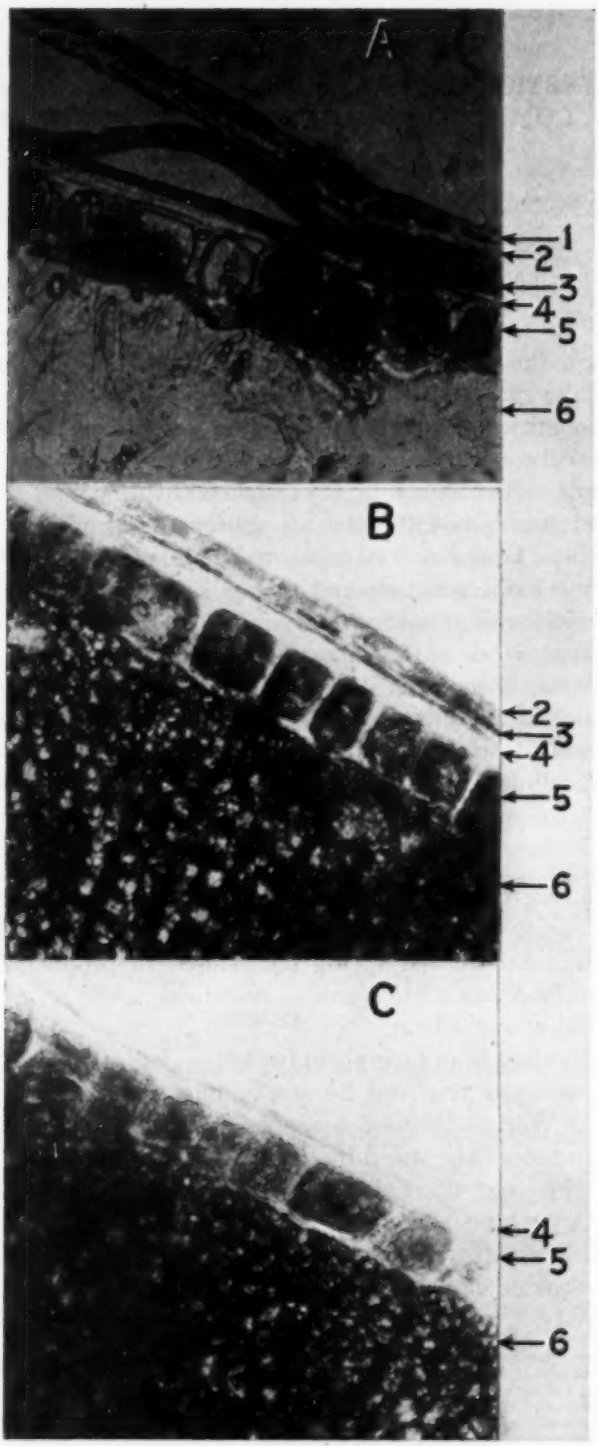

Fig. 1. Photomicrographs of wheat cross sections after successive removal of bran layers. Magnified about 215 times.
A. Original wheat. B. After removal of epidermis. C. After removal of cross layers and testa.
1. Epidermis. 2. Cross layer cells. 3. Testa layers. 4. Hyaline.
5. Aleurone. 6. Starchy endosperm.

then separated by stirring the grain with more 95% ethyl alcohol in the Waring Blendor at reduced speed. Each preparation was removed by filtration and air dried. Microscopic examination of the grain showed that the bran layers were removed to the hyaline layer (Figure 1).

The de-branned wheat was placed in a large Buechner funnel and washed once with 95% alcohol, then once with water, followed by a wash with dilute hydrochloric acid, and finally by another water wash before being air dried.

After air drying, the de-branned wheat was tempered to a moisture content of 16% and milled on an experimental mill to obtain samples of endosperm and of aleurone with the adhering hyaline. The latter preparation was contaminated with considerable endosperm, and, consequently, corrections, based upon its starch content, were applied to the determinations.

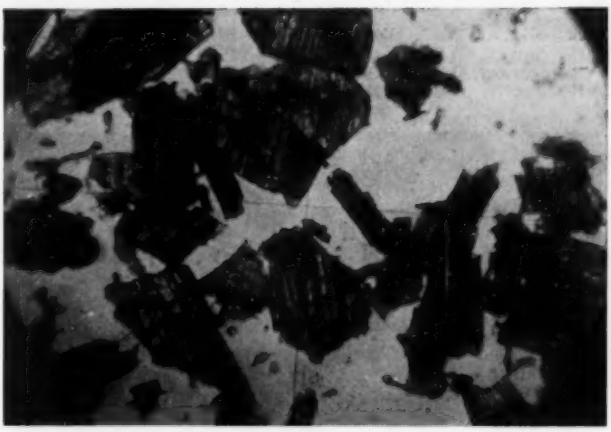

Fig. 2. Photomicrograph of epidermis preparation. Only epidermis cells appear to be present. Magnified about 50 diameters.

Microscopic examination of the various preparations (Figures 2, 3, and 4) indicated that the testa was contaminated with cross layer and epidermis cells. Consequently, a correction was made as follows. A small amount of the dry preparation was placed on a blood-counting chamber, and evenly distributed with a spatula. Then the length and breadth of every particle occurring within the marked area were measured with a microscope containing a scale in the eyepiece. A thickness factor for each layer was determined by measuring the relative thickness of each layer in cross section of wheat grains with the same scaled eyepiece. These three factors were then multiplied together to obtain the relative volume of each particle. The sum of the volumes for each of the three fractions divided by the total volume

was assumed to represent the percentage of the fraction present. The results of the testa fraction were then corrected for the percentage of each contaminating layer found by this procedure.

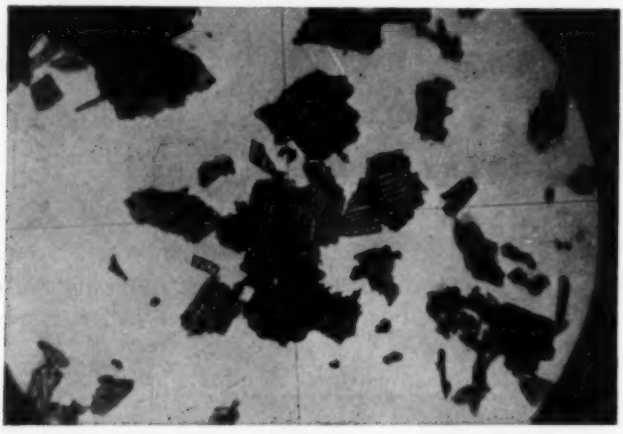

Fig. 3. Photomicrograph of cross layer preparation. Only cross layer cells are present. Magnified about 50 diameters.

Fig. 4. Photomicrograph of testa preparation. Epidermis cells (a), cross layer cells (b), and testa cells (c) are present in the preparation. Magnified about 50 diameters.

To check the operations during the removal of various layers, proximate analyses were made on the whole wheat and also upon the portion left after removal of each layer. The latter figures were compared with a calculated value which the preparation should have on the basis of the proximate analysis of the removed layer.

Moisture, protein, total ash, crude fat (ether extract), crude fiber, pentosans, and starch (by Rask's procedure) were determined as described in *Cereal Laboratory Methods* (4th ed., 1941). Cellulose was determined by the method of Crampton and Maynard (1938).

Results

The percentages of each layer found in whole wheat and bran after making the corrections described are shown in Table I.

TABLE I

PERCENTAGE DISTRIBUTION OF TOTAL DRY MATTER OF BRAN AMONG THE INDIVIDUAL LAYERS

	Sample 1		Sample 2	
	Per cent of whole wheat	Per cent of bran	Per cent of whole wheat	Per cent of bran
Epidermis	3.9	26.7	3.9	27.1
Cross layer	0.9	6.2	0.9	6.2
Testa	0.5	3.4	0.7	4.9
Aleurone and hyaline	9.3	63.7	8.9	61.8
Total	14.6	100.0	14.4	100.0

The epidermis and the hyaline-aleurone preparation constitute the majority of the dry matter of the bran (about 27% and 62% respectively). The amounts of testa and cross layers are comparatively small. The sum of the percentages of the individual bran layers indicates the percentage of bran in wheat. The brans of these wheats amounted to about 14.5% of the kernel. This figure corresponds with that given by the Wheat Flour Institute (1940), but is considerably higher than the value of 8% given by Briggs (1935). The experimental values are also somewhat higher than those suggested by Miller (1941) who estimates the content to be somewhat over 12%. Jago and Jago (1921) give the following figures from Mege Mouris:

	Percentage of entire kernel
	%
Epicarp	0.5
Mesocarp	1.0
Endocarp	1.5
Testa and hyaline	2.0

The epicarp and mesocarp taken together correspond to the portion referred to in this paper as epidermis, and the endocarp corresponds to the cross layer fraction. While the amount of the epidermis found in this study was greater than that reported by Mege Mouris, his figure for the cross layer fraction is higher. The hyaline layer was not separated from the aleurone layer in the present work and therefore it is not possible to compare directly his estimation for the constructed percentage of testa and hyaline.

If the figure of 2% for testa and hyaline given by Mege Mouris is assumed to be correct, then by subtracting the average value found for testa in the present work, the figure of 1.4% is obtained for the

TABLE II

PROXIMATE CHEMICAL COMPOSITION OF THE DIFFERENT PARTS OF THE WHEAT KERNEL [1]

Constituents	Whole wheat					Bran layers					Hand-picked hyaline	Starchy endosperm		Hand-picked starchy endosperm
	Original	Minus epidermis		Minus epidermis, cross layers, testa		Epidermis	Cross layers	Testa	Hyaline-aleurone	Hand-picked hyaline-aleurone		Endosperm		Endosperm
		Anal.	Calc.	Anal.	Calc.							Anal.	Calc.	
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Total ash														
Sample 1	1.87	1.88	2.19	1.29	1.62	1.41	13.8	19.3	4.9	—	—	0.70	0.92	—
Sample 2	1.88	1.95	1.90	1.31	1.69	1.35	15.7	23.5	5.7	—	1.98	0.74	0.90	—
Sample 3	2.03	—	—	—	—	1.38	13.1	14.6	7.5	—	—	0.66	—	—
Sample 4	2.24	—	—	—	—	1.53	12.6	14.6	12.6	13.3	—	0.70	—	0.60
Crude protein														
Sample 1	15.0	15.7	15.9	15.6	15.8	5.2	10.9	13.9	33.4	—	—	13.9	13.6	—
Sample 2	16.1	16.4	16.5	16.2	16.4	3.6	11.2	22.7	37.6	—	19.5	14.2	13.8	—
Sample 3	11.4	—	—	—	—	—	—	—	30.6	—	—	8.5	—	—
Sample 4	14.1	—	—	—	—	—	—	—	29.3	29.5	—	8.7	—	10.3
Crude fat														
Sample 1 [2]	2.2	2.3	2.2	2.0	2.3	1.2	0.6	0.0	8.8	—	—	1.0	1.3	—
Sample 2 [2]	2.3	2.3	—	—	2.4	0.8	0.4	0.2	7.0	—	—	1.0	0.9	—
Sample 2 [3]	3.0	—	2.4	1.4	—	—	—	—	10.6	—	—	—	—	—
Sample 3 [3]	3.1	—	—	—	—	—	—	—	11.5	—	—	1.9	—	—
Sample 4 [3]	3.1	—	—	—	—	—	—	—	8.8	—	—	2.2	—	2.8

[1] Moisture-free basis.
[2] Ether extract method.
[3] Acid hydrolysis method.

TABLE II—*Continued*

Constituents	Whole wheat					Bran layers					Hand-picked hyaline	Starchy endosperm		Hand-picked starchy endosperm
	Original	Minus epidermis		Minus epidermis, cross layers, testa		Epi-dermis	Cross layers	Testa	Hyaline-aleurone	Hand-picked hyaline-aleurone		Endosperm		Endosperm
		Anal.	Calc.	Anal.	Calc.							Anal.	Calc.	
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Crude fiber														
Sample 1	3.1	3.0	2.3	1.0	1.5	28.2	21.4	1.2	6.1	—	—	0.3	0.4	—
Sample 2	2.6	1.6	1.9	1.0	1.4	27.1	19.9	1.4	7.0	—	—	0.2	0.4	—
Cellulose														
Sample 1	2.5	1.4	1.5	1.0	1.3	32.4	22.8	0.0	6.5	—	—	0.3	0.4	—
Sample 2	2.1	1.7	1.4	1.0	1.1	31.8	22.9	0.0	6.5	—	7.0	0.3	0.4	—
Sample 3	2.2	—	—	—	—	—	—	—	5.1	—	—	0.2	—	—
Sample 4	2.6	—	—	—	—	—	—	—	4.2	3.3	—	0.2	—	0.2
Pentosans														
Sample 1	6.5	5.6	5.7	5.4	5.2	34.0	30.3	18.2	33.7	—	—	3.5	4.4	—
Sample 2	6.9	5.8	6.0	5.3	5.6	37.9	29.2	16.0	25.9	—	46.1	3.5	3.1	—
Sample 3	7.2	—	—	—	—	—	—	—	30.9	—	—	3.0	—	—
Sample 4	7.8	—	—	—	—	—	—	—	25.8	27.5	—	3.2	—	3.3
Starch														
Sample 1	63.1	—	—	—	—	—	—	—	—	—	—	78.9	—	—
Sample 2	61.3	—	—	—	—	—	—	—	—	—	—	78.1	—	—
Sample 3	68.4	—	—	—	—	—	—	—	—	—	—	82.7	—	80.2
Sample 4	64.1	—	—	—	—	—	—	—	—	—	—	83.8	—	—

hyaline layer. By subtracting this value from the average value obtained for the hyaline-aleurone preparation, the figure of 7.7% is obtained for the aleurone layer. The total of the layers reported upon by Mege Mouris amounts to 5%. From the figure given by Briggs for bran, this would only allow the aleurone to be about 3% of the whole wheat, a much lower value than that found in this study.

Results of the proximate chemical analyses are shown in Table II.

The distribution of the different constituents in the individual bran layers and in the starchy endosperm were calculated from the data recorded in Tables I and II and are summarized in Tables III and IV.

TABLE III

AVERAGE PERCENTAGE DISTRIBUTION OF ASH, PROTEIN, FAT, CRUDE FIBER, CELLULOSE, AND PENTOSANS OF THE TOTAL BRAN FOUND IN THE INDIVIDUAL BRAN LAYERS [1]

Layer	Ash	Protein	Crude fat	Crude fiber	Cellulose	Pentosans
	%	%	%	%	%	%
Epidermis	6.8	4.8	4.1	58.1	61.2	34.8
Cross layers	16.5	2.6	0.6	9.6	9.7	6.4
Testa	16.2	2.9	0.1	0.0	0.0	2.4
Hyaline and aleurone	60.5	89.7	94.2	32.3	29.1	56.4

[1] Data for samples 1 and 2 averaged together. Obtained by calculation from Tables I and II.

TABLE IV

AVERAGE PERCENTAGE DISTRIBUTION OF ASH, PROTEIN, FAT, CRUDE FIBER, CELLULOSE, AND PENTOSANS OF THE TOTAL GRAIN FOUND IN DIFFERENT PARTS OF THE WHEAT KERNEL [1]

Layer	Ash	Protein	Crude fat	Crude fiber	Cellulose	Pentosans
	%	%	%	%	%	%
Epidermis	2.8	1.2	1.8	37.8	55.2	20.8
Cross layers	7.0	0.6	0.2	6.2	8.7	3.8
Testa	7.0	0.7	0.0	0.0	0.0	1.4
Hyaline-aleurone	25.7	20.8	32.0	21.0	26.2	33.8
Starchy endosperm	32.8	77.3	38.6	7.7	12.1	44.8
TOTAL	75.3	100.6	72.8	76.7	102.2	104.6

[1] Data for samples 1 and 2 averaged together.

Ash. The results in Table II present a fairly clear picture of the ash content of the bran layers. Since both the cross layer and testa fractions had absorbed sodium hydroxide in the removal process, the ash contents of these fractions were corrected by making sodium determinations using the uranyl acetate precipitation method (A. O. A. C. official method, 1940). On the basis of preliminary work the preparations were assumed naturally to contain 1,000 p.p.m. sodium and the excess sodium contents, calculated as sodium oxide, were deducted from the original ash to give the figures recorded in Table II.

The average ash content of the various fractions is as follows:

	%
Epidermis	1.4
Cross layer	13.0
Testa	18.0
Aleurone-hyaline preparation	6.0
Endosperm (starchy)	0.7

The ash content of the hyaline-aleurone preparation is of the same order as that of whole bran, while the ash content of the endosperm corresponds to that of clear flour. The ash contents of the cross layer and testa fractions are very high.

The variation in the ash contents of the testa and hyaline-aleurone fractions of the different wheat samples was quite large. The ash content of the hyaline-aleurone of sample 4 was especially high, but this figure was verified in the corresponding hand-picked sample. However, because of the small number of samples, it is not possible to state any definite trend in wheat types. The ash content of one sample (No. 4) of hand-picked hyaline layer was much lower than that of the corresponding hyaline-aleurone sample, indicating that the ash of the latter is concentrated largely in the aleurone layer. The ash contents of the aleurone layers should then be higher than those recorded for the hyaline-aleurone combinations.

As shown in Tables III and IV, the hyaline-aleurone preparation contains almost 60% of the total ash of the bran, and about 25% of the ash of the whole wheat.

Crude Protein. The average protein contents of the different bran layers expressed on the dry basis are approximately as follows:

	%
Epidermis	4
Cross layer	11
Testa	18
Hyaline-aleurone preparation	33
Starchy endosperm	11

These data were verified by a qualitative microchemical study *in situ* in which the testa layer and the aleurone cell contents were shown to contain considerable protein. One of the most striking facts is the high protein content of the hyaline-aleurone preparation. Since the protein content of the sample of hyaline (which was shown microscopically still to contain some aleurone cells) is lower than that of the corresponding hyaline-aleurone combination, the protein content of the aleurone layer must be higher than those recorded for the hyaline-aleurone preparations.

The hyaline-aleurone preparation constitutes about 90% of the protein of the whole bran, and slightly over 20% of the protein of the whole wheat.

Crude Fat. Of the bran layers, only the hyaline-aleurone preparation contains more than a negligible amount of crude fat. Of the two layers, qualitative microchemical studies indicated that the aleurone probably contains the bulk of this constituent. The acid hydrolysis method for fat yields considerably more fat from the hyaline-aleurone preparation than does the ether extraction method. This is perhaps due to the heavy cell walls of the aleurone which are hydrolyzed by the former method. The hyaline-aleurone preparation contains about 94% of the ether extract of the bran, and about 32% of the ether extract of the whole grain, while the starchy endosperm contains about 38% of the ether extract of the whole grain.

Crude Fiber and Cellulose. As one would suspect, the pictures for crude fiber and cellulose are roughly parallel to each other. The average percentages of crude fiber and cellulose are as follows in round numbers:

	Crude fiber %	Cellulose %
Epidermis	28	32
Cross layer	21	23
Testa	1.3	0.0
Hyaline-aleurone preparation	6.5	6.5
Endosperm (starchy)	0.3	0.3

These data were again verified by qualitative microchemical data which indicated that cellulose is high in the epidermis, cross layers, and hyaline cell walls, present in the aleurone cell walls, but almost nonexistent in the testa layer. In the case of white wheat, however, microchemical studies indicated that a small amount of cellulose does occur in the cell walls of the testa. The apparent absence of this constituent in the testa may be due to the inaccuracy of the correction procedure as previously described.

Table III shows that the epidermis contains 61% and the hyaline-aleurone preparation 29% of the cellulose in bran. When referred to the whole grain, the epidermis contains 55%, the hyaline-aleurone preparation 25%, and the endosperm 12% of the cellulose.

Pentosans. The pentosans seem to be uniformly distributed throughout the bran, as can be seen from the following average results:

	%
Epidermis	35
Cross layer	30
Testa	17
Hyaline-aleurone preparation	30
Starchy endosperm	3.5

It is noteworthy that the sample of hand-picked hyaline was very high in pentosan content.

The epidermis contains 35% and the hyaline-aleurone preparation 56% of the pentosan content of the bran (Table III), whereas the epidermis contains 21%, the hyaline-aleurone preparation 34%, and the endosperm about 44% of the pentosans of the whole wheat (Table IV).

Discussion

The results indicate that the nutritive value of bran resides largely in the hyaline-aleurone preparation, as this fraction contains the bulk of the ash, protein, and fat of the bran, and a large portion of the same constituents in whole wheat. The other layers contain little nutritive value, but the epidermis and cross layers would serve as a good source of furfural because of their high pentosan content.

Over 50% of the mineral content of whole wheat is contained in bran. Comparison of the proximate chemical analysis of the residual wheat after removal of the epidermis, cross layer, and testa, and the starchy endosperm recorded in Table II, with the calculated values indicates that some ash was lost in the process of removing the cross layer and testa cells. On the other hand, the experimental values for protein agree with the calculated ones within experimental error. For many of the other constituents, this is also true.

The sum of the ash, protein, fat, cellulose, and pentosan percentages for each individual layer is less than 100. Cutin, suberin, pectin compounds, phytin, and lignin were not determined, but were present in some layers as indicated by microchemical tests.

Summary

The various layers of wheat bran were separated by a physical and chemical process, and subjected to proximate chemical analysis. The total bran was found to be about 14.5% of the whole wheat, of which about 3.9% was epidermis, 0.9% cross layers, 0.6% testa, and 9.0% hyaline and aleurone. Wheat epidermis contained about 1.4% ash, 4% protein, 1% fat, 32% cellulose, and 35% pentosans. The cross layers contained about 13% ash, 11% protein, 0.5% fat, 23% cellulose, and 30% pentosans. The testa layers contained about 18% ash, 15% protein, no cellulose, no fat, and 17% pentosans. A hyaline-aleurone preparation contained about 5% ash, 35% protein, 7% fat, 6% cellulose, and 30% pentosans.

The starchy endosperm contained about 0.7% ash, 14% protein, 1% fat, 0.3% cellulose, and 3.5% pentosans.

The hyaline-aleurone preparation contained about 60% of the ash, 90% of the protein, 94% of the fat, 56% of the pentosans, and 29% of the cellulose in whole bran. All figures are expressed on the dry basis.

Of the botanical layers which occur in commercial bran, the aleurone layer appears to be of paramount importance from a nutritive viewpoint.

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MULTIPLE BLEACHING OF FLOUR. IV. BLEACHING EFFICIENCY IN SYSTEMS INVOLVING CHLORINE DIOXIDE AND ANOTHER BLEACHING GAS[1]

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The purpose of this paper is to report experimental results which show the best way to utilize chlorine dioxide in combination with another bleaching gas in the multiple bleaching of flour.

In recent years much interest has been evidenced in the evaluation of chlorine dioxide as a flour bleaching reagent. This reagent was shown by Ferrari, Hutchinson, Croze, and Mecham (1941) to be quite similar in its flour bleaching action to nitrogen trichloride. To bring about a given amount of color removal or to mature a flour from the

[1] Paper No. 70, Journal Series, General Mills, Inc., Research Department.
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baking standpoint, only from one-half to two-thirds as much chlorine dioxide is needed as in the case of nitrogen trichloride. Until recently the methods for making chlorine dioxide were inadequate for providing the fine control needed in applying this gas for flour bleaching purposes. However, commercial equipment has now been manufactured by the Mathieson Alkali Works, Inc., based on the process developed by Hutchinson and Mecham (1943). Adequate control of chlorine dioxide for flour bleaching purposes can be obtained by this method.

The first three papers of this series by the present authors and Mecham (1945) dealt with repeated bleaching treatments of flour with a single gaseous reagent and the effects of agitation on bleaching efficiency, multiple bleaching with nitrogen peroxide and another gas, and multiple bleaching with nitrogen trichloride and another gas, respectively. Multiple bleaching has been defined as the treatment of flour by repeated applications of a single gaseous reagent or by the simultaneous or sequential treatment of flour with two or more gaseous bleaching reagents.

Some multiple bleaching treatments involving the use of chlorine dioxide with such other bleaching gases as chlorine, hypochlorous acid, nitrogen trichloride, and nitrogen peroxide have been covered by a patent to Ferrari and Hutchinson (1943).

Apparatus and Procedure

The agitating equipment and methods for the generation and metering of the bleaching gases have been briefly described by Ferrari, Hutchinson, and Mecham (1945), in the first paper of this series and are dealt with in more detail by Hutchinson, Derby, and Ferrari (1947).

Experimental

Multiple Bleaching with Chlorine Dioxide and Chlorine. Experiments were conducted in which chlorine dioxide and chlorine were used in combination in the batch scale bleaching of patent flour. In order to ascertain the best way in which to utilize these reagents in multiple bleaching treatments, they were applied to the flour both simultaneously and sequentially. Typical examples of such treatments are shown in Table I. It is seen that a substantial increase in color removal is gained by applying the chlorine to the flour first, followed by the chlorine dioxide. The application of the two reagents in the reverse order or simultaneously resulted in much less color removal. Judging from an extensive experience with many flours, it is probable that it would take between 0.016 and 0.032 ounces of benzoyl peroxide per barrel to reduce the carotene content of this flour from 0.98 to 0.80 p.p.m. From the economic standpoint such a saving in benzoyl

peroxide due to the application of chlorine and chlorine dioxide in the preferred order is quite significant.

Although some difference in effect on pH may be noted in Table I due to the method of application of the two gases, it is not regarded as significant.

TABLE I

CHLORINE DIOXIDE AND CHLORINE APPLIED TO A PATENT FLOUR SIMULTANEOUSLY OR SEQUENTIALLY

Bleaching treatment per bbl flour	pH	Carotene [1] p.p.m.
Unbleached patent flour	6.01	2.20
2 g NCl_3 plus 0.048 oz benzoyl peroxide	6.03	0.66
0.8 g ClO_2	6.03	1.27
21 g Cl_2	5.91	1.32
Simultaneous: 0.8 g ClO_2 and 21 g Cl_2	5.84	0.98
Sequential: 0.8 g ClO_2 followed by 21 g Cl_2	5.81	0.99
21 g Cl_2 followed by 0.8 g ClO_2	5.74	0.80

[1] Flour pigment content expressed as carotene.

From the baking standpoint this bleaching combination offers some interesting possibilities due to the lowering of pH by the chlorine and the maturing action of the chlorine dioxide. Practically all of the flour samples which have been subjected to multiple bleaching treatments have been baked in one or more experimental or commercial baking procedures. Generally speaking, it has been found that the flour bleached with the preferred order of application of the reagents from the color removal standpoint has possessed at least as good baking characteristics as flours bleached with the same quantities of the same reagents simultaneously or in the reverse order of application.

Multiple Bleaching with Chlorine Dioxide and Hypochlorous Acid. In the bleaching of flour with chlorine dioxide and hypochlorous acid, the greatest color removal is realized when the hypochlorous acid is applied first, followed by the chlorine dioxide. The reverse order of application of these reagents brings about less color removal. Typical examples illustrating these facts are shown in Table II. It can be seen that the advantage due to the preferred order of application of the reagents is rather slight in the case of the patent flour which was bleached by the two gases to the level of about 0.75 p.p.m. of residual carotene. In contrast to this result is the rather large difference in color removal with the two orders of application of chlorine dioxide and hypochlorous acid on the first clear flour shown in Table II. With some reagent combinations the advantages of the preferred order of application become quite slight at low residual carotene values in the bleached flour, although they are pronounced at high residual carotene values. With other reagent combinations the effects are pronounced at both high and low residual carotene values in the bleached flour.

TABLE II

FLOUR BLEACHING WITH CHLORINE DIOXIDE AND HYPOCHLOROUS ACID IN BATCH SCALE EQUIPMENT—EFFECT OF ORDER OF APPLICATION ON COLOR REMOVAL

Bleaching treatment per bbl flour	Carotene p.p.m.
Unbleached patent flour	2.41
2 g NCl_3 plus 0.048 oz benzoyl peroxide	0.75
1.1 g ClO_2	1.21
20 g HOCl	1.27
1.1 g ClO_2 followed by 20 g HOCl	0.77
20 g HOCl followed by 1.1 g ClO_2	0.75
Unbleached first clear flour	2.94
5 g NCl_3	1.20
1.1 g ClO_2	2.32
1.4 g ClO_2	2.08
1.1 g ClO_2 followed by 15 g HOCl	2.27
15 g HOCl followed by 1.1 g ClO_2	1.97
1.4 g ClO_2 followed by 25 g HOCl	1.65
25 g HOCl followed by 1.4 g ClO_2	1.37

In papers I and II of this series, it has been shown that chlorine dioxide is preferably applied to the flour first, followed by nitrogen trichloride or nitrogen peroxide, to achieve the greatest color removal effect.

Summary

The preferred orders of application of chlorine dioxide and other gaseous reagents in the multiple bleaching of flour are as follows:

Chlorine dioxide followed by nitrogen trichloride
Chlorine dioxide followed by nitrogen peroxide
Hypochlorous acid followed by chlorine dioxide
Chlorine followed by chlorine dioxide

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MULTIPLE BLEACHING OF FLOUR. V. BLEACHING EFFICIENCY IN SYSTEMS INVOLVING CHLORINE AND HYPOCHLOROUS ACID OR THREE GASEOUS REAGENTS [1]

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The purposes of this paper are to submit data on the bleaching of flour with various combinations of three gaseous reagents selected from the group consisting of chlorine, chlorine dioxide, nitrogen trichloride, and nitrogen peroxide; to show the effects of blending two flours subjected to multiple bleaching treatments with different combinations of gaseous reagents; and to summarize and integrate the data submitted in this series of papers on the multiple bleaching of flour with gaseous reagents.

Multiple flour bleaching treatments involving chlorine and nitrogen peroxide, nitrogen trichloride, and chlorine dioxide have been described in papers II, III, and IV, respectively, of this series by the present authors (1945, 1945, and 1947). In this report results with the combination of chlorine and hypochlorous acid will be considered.

Since such remarkable and unexpected results were obtained by multiple flour bleaching treatments involving two gaseous reagents, it was decided to ascertain the feasibility of using multiple bleaching treatments involving three gaseous reagents applied to the same flour. The blending of samples of the same flour bleached with multiple bleaching treatments involving different combinations of reagents appeared to offer more bleaching flexibility, particularly from the baking standpoint, and was accordingly investigated. Such blended flours may involve three or four gaseous reagents.

Some of the multiple bleaching treatments described in this paper are covered by patents issued to Ferrari and Hutchinson (1943 and 1944).

Apparatus and Procedures

Methods for the generation and metering of the bleaching gases and the agitating equipment have been briefly described in the first paper of this series (Ferrari, Hutchinson, and Mecham, 1945). They are described in greater detail by Hutchinson, Derby, and Ferrari (1947).

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Multiple Bleaching with Chlorine and Hypochlorous Acid. Flour bleaching with combinations of chlorine and hypochlorous acid is best carried out by applying the two reagents in sequence rather than simultaneously. Examples of such treatments are shown in Table I.

TABLE I

CHLORINE AND HYPOCHLOROUS ACID APPLIED TO A PATENT FLOUR SIMULTANEOUSLY OR SEQUENTIALLY IN BATCH SCALE APPARATUS

Description of bleaching treatment per bbl flour	Pekar or slick score	Carotene [1] p.p.m.
Unbleached	Creamy yellow	2.84
2½ g NCl_3 plus 0.32 oz benzoyl peroxide	10	0.85
25 g HOCl	8 Creamy	1.48
0.75 oz Cl_2	—	1.63
Simultaneously: 0.75 oz Cl_2 plus 25 g HOCl	8 Creamy	1.32
Sequentially: 25 g HOCl followed by 0.75 oz Cl_2	9 Slightly creamy	1.10
Sequentially: 0.75 oz Cl_2 followed by 25 g HOCl	9 Slightly creamy	1.08

[1] Flour pigment content expressed as carotene.

The order of application of the two reagents is immaterial, but either sequence is highly preferable to the simultaneous application of the two reagents. A substantial quantity of benzoyl peroxide, probably between 0.016 and 0.032 ounces per barrel of flour, would be needed to lower the carotene content from 1.32 to 1.10 p.p.m.; so the practical importance of applying chlorine and hypochlorous acid in sequence is quite apparent.

Multiple Bleaching with Three Gaseous Reagents. In the preceding papers of this series on the multiple bleaching of flour with gaseous reagents many different combinations of two reagents have been pointed out which produce excellent bleaching results from the color removal standpoint. The great flexibility in the bleaching process resulting from these many reagent combinations has greatly increased the possibilities of achieving optimum baking properties through the proper choice of reagents for each different flour.

As chlorine, nitrogen trichloride, chlorine dioxide, and nitrogen peroxide all have somewhat different effects on the baking properties of flour, it was thought that flexibility in bleaching treatments might be increased still more by the application of three gaseous reagents to the same flour.

Four possible ways of applying any three gaseous reagents to flour are as follows:

1. Simultaneous application of the three gases.
2. Sequential treatment with the three gases (six possible combinations).

3. Application of one gas followed by a simultaneous treatment with the other two gases (three possible combinations).
4. Simultaneous application of two gases followed by a treatment with the third gas (three possible combinations).

Thirteen different treatments are, therefore, possible for the application of any three gaseous reagents to flour.

Since the results with the simultaneous treatment of flour with two gases were found so uniformly poor compared to a preferred order of application of combinations of two gaseous bleaching reagents, the simultaneous treatment of flour with three gaseous reagents was not tried. However, methods 2, 3, and 4 above were all made the subject of extended experimentation.

TABLE II

BLEACHING OF PATENT FLOUR WITH CHLORINE DIOXIDE, NITROGEN TRICHLORIDE, AND NITROGEN PEROXIDE APPLIED SEQUENTIALLY IN BATCH SCALE EQUIPMENT

Bleaching treatment per bbl flour	Carotene p.p.m.
Unbleached flour	2.20
0.8 g ClO_2 followed by 2 g NCl_3 followed by 1.5 g NO_2	0.63
Sample after 0.8 g ClO_2 alone	1.42
Sample after 0.8 g ClO_2 followed by 2 g NCl_3	0.69
0.8 g ClO_2 followed by 1.5 g NO_2 followed by 2 g NCl_3	0.68
Sample after 0.8 g ClO_2 alone	1.38
Sample after 0.8 g ClO_2 followed by 1.5 g NO_2	0.86
0.8 g ClO_2 followed by 1.5 g NO_2 followed by 0.75 oz Cl_2	0.82
Sample after 0.8 g ClO_2 alone	1.36
Sample after 0.8 g ClO_2 followed by 1.5 g NO_2	0.86
0.8 g ClO_2 followed by 0.75 oz Cl_2 followed by 1.5 g NO_2	0.78
Sample after 0.8 g ClO_2 alone	1.40
Sample after 0.8 g ClO_2 followed by 0.75 oz Cl_2	1.01

Typical examples of method 2 are shown in Tables II and III. In Table II several things may be noted. First it can be seen that chlorine dioxide was applied first in each of the bleaching treatments shown. The accuracy of the application of the chlorine dioxide treatments is shown in the residual carotene contents of 1.42, 1.38, 1.36, and 1.40 p.p.m. for the four different applications. The two cases in which 1.5 g nitrogen peroxide was applied following the chlorine dioxide both resulted in residual carotene values of 0.86 p.p.m., again showing the fine control that was achieved in the experimental bleaching techniques used.

In Table II it is seen that chlorine dioxide followed by nitrogen trichloride followed by nitrogen peroxide brought about more color

removal than chlorine dioxide followed by nitrogen peroxide followed by nitrogen trichloride. Also the application of chlorine dioxide followed by chlorine followed by nitrogen peroxide was more effective from the color removal standpoint than chlorine dioxide followed by nitrogen peroxide followed by chlorine. Table III shows that treatment of flour with nitrogen trichloride followed by chlorine followed by nitrogen peroxide was more effective than the application of nitrogen trichloride followed by nitrogen peroxide followed by chlorine. From these examples the generalization may apparently be made that

TABLE III

BLEACHING OF PATENT FLOUR WITH NITROGEN TRICHLORIDE, NITROGEN PEROXIDE, AND CHLORINE APPLIED SEQUENTIALLY IN BATCH SCALE APPARATUS

Bleaching treatment per bbl flour	Carotene p.p.m.
2 g NCl_3 followed by 1.5 g NO_2 followed by 0.75 oz Cl_2	0.75
Sample after 2 g NCl_3 alone	1.08
Sample after 2 g NCl_3 followed by 0.75 oz Cl_2	0.80
2 g NCl_3 followed by 0.75 oz Cl_2 followed by 1.5 g NO_2	0.69
Sample after 2 g NCl_3 alone	1.07
Sample after 2 g NCl_3 followed by 1.5 g NO_2	0.86
0.75 oz Cl_2 followed by 2 g NCl_3 followed by 1.5 g NO_2	0.69
Sample after 0.75 oz Cl_2 followed by 2 g NCl_3	0.77
0.75 oz Cl_2 followed by 1.5 g NO_2 followed by 2 g NCl_3	0.78
Sample after 0.75 oz Cl_2 followed by 1.5 g NO_2	0.89
1.5 g NO_2 followed by 0.75 oz Cl_2 followed by 2 g NCl_3	0.86
Sample after 1.5 g NO_2 followed by 0.75 oz Cl_2	1.01
1.5 g NO_2 followed by 2 g NCl_3 followed by 0.75 oz Cl_2	0.86
Sample after 1.5 g NO_2 followed by 2 g NCl_3	0.92
Unbleached flour	2.20

in the application of three different gaseous reagents to flour in sequence, the last two reagents should be applied in the order of application found best from the color removal standpoint in the treatment of flour with these two reagents alone.

In Table III six different sequential applications of 0.75 ounces chlorine, 2 g nitrogen trichloride, and 1.5 g nitrogen peroxide per barrel of flour are shown. The results indicate that the nitrogen peroxide should be applied last and that it makes little difference whether nitrogen trichloride is applied first followed by chlorine or chlorine is applied first followed by the nitrogen trichloride.

It will be noted that the color removal achieved by the third reagent in the examples shown in Tables II and III is relatively small, although

it is significant at the low levels of residual carotene content involved. There is much doubt as to whether the third gas could be justified commercially to secure so little additional color removal.

Examples of the application of one gaseous reagent to flour followed by the simultaneous treatment of the same flour with two different gaseous bleaching agents are shown in Table IV. Results achieved

TABLE IV

BLEACHING OF PATENT FLOUR WITH A GASEOUS REAGENT FOLLOWED BY TWO OTHER DIFFERENT GASEOUS REAGENTS APPLIED SIMULTANEOUSLY IN BATCH SCALE EQUIPMENT

Bleaching treatment per bbl flour	Carotene p.p.m.
2 g NCl_3 followed by 1.5 g NO_2 and 0.5 oz Cl_2—applied simultaneously	0.80
Sample after first reagent above	1.10
2 g NCl_3 followed by 1.5 g NO_2 and 0.75 oz Cl_2—applied simultaneously	0.75
Sample after first reagent above	1.10
0.8 g ClO_2 followed by 1.5 g NO_2 and 0.5 oz Cl_2—applied simultaneously	0.82
1.5 g NO_2 followed by 0.5 oz Cl_2 and 0.8 g ClO_2 applied simultaneously	0.96
0.8 g ClO_2 followed by 1.5 g NO_2 and 0.75 oz Cl_2—applied simultaneously	0.86
1.5 g NO_2 followed by 0.8 g ClO_2 and 0.75 oz Cl_2—applied simultaneously	0.89
Unbleached flour	2.20

show about the same residual carotene content in the flour as can be realized from the treatment of flour with only two gases applied in the preferred order of application from the color removal standpoint. Consequently, this three gas treatment is not considered to have much promise. Again in Table IV it is illustrated that nitrogen peroxide should not be applied to flour first in multiple bleaching treatments.

TABLE V

BLEACHING OF PATENT FLOUR BY THE SIMULTANEOUS APPLICATION OF TWO GASEOUS REAGENTS FOLLOWED BY A THIRD GASEOUS REAGENT IN BATCH SCALE BLEACHING APPARATUS

Bleaching treatment per bbl flour	Carotene p.p.m.
0.8 g ClO_2 and 0.5 oz Cl_2 simultaneously followed by 1.5 g NO_2	0.80
0.8 g ClO_2 and 0.75 oz Cl_2 simultaneously followed by 1.5 g NO_2	0.78
2.0 g NCl_3 and 0.75 oz Cl_2 simultaneously followed by 1.5 g NO_2	0.80
2.5 g NCl_3 and 0.50 oz Cl_2 simultaneously followed by 1.5 g NO_2	0.77
0.75 oz Cl_2 and 1.5 g NO_2 simultaneously followed by 2.0 g NCl_3	0.74
0.75 oz Cl_2 and 1.5 g NO_2 simultaneously followed by 0.8 g ClO_2	0.69
Unbleached flour	2.20

Bleaching of flour with two different gases simultaneously, followed by treatment with a third gaseous reagent, is shown by several examples in Table V. Generally speaking, this method of bleaching with three gaseous reagents is not promising since the color removal achieved does not appear to be substantially greater than that which would be realized by the treatment of the flour with two reagents in their pre-

ferred order of application from the color removal standpoint. It may therefore be concluded that the use of three gaseous bleaching reagents on flour does not seem practical from the color removal standpoint when they are applied in any of the ways previously listed and illustrated. Numerous bakings of these samples and other similar ones bleached with three reagents indicated that no advantage nor any greater flexibility was gained from the baking standpoint in these three gas treatments.

The flexibility and advantages sought but not achieved in three-reagent bleaching were found to be obtainable by bleaching different aliquots of a given flour with two different combinations of two gaseous reagents and then blending the resulting bleached flours. An example of this type of treatment, which is capable of producing quite surprising results, is shown in Table VI. In this case, both portions

TABLE VI

BLENDING OF TWO PORTIONS OF THE SAME FLOUR BLEACHED WITH DIFFERENT GASEOUS REAGENT MULTIPLE BLEACHING COMBINATIONS

Flour sample	Bleaching treatment per bbl flour	Bread characteristics			Carotene p.p.m.
		Crumb color	Crumb grain	Loaf volume	
				cc	
1	2.5 g NCl_3 plus 0.032 oz benzoyl peroxide	10	10	2,800	0.80
2	1.75 oz Cl_2 followed by 1.5 g NO_2	10 dull white	10	2,670	0.87
3	3.0 g NCl_3 followed by 1.5 g NO_2	10 dull creamy	10	2,655	0.78
4	Blend of 50% sample 2 and 50% sample 3	11	10+	2,795	0.83

of the flour were bleached with nitrogen peroxide, the cheapest and most economical of gaseous reagents. Part of the flour had its baking properties influenced by chlorine, part by nitrogen trichloride, and the effects were supplementary in character as can readily be seen by the baking results of the blended flour. This type of multiple bleaching treatment is cheap and provides great flexibility, resulting in optimum baking characteristics in the bleached flour when applied correctly. It can of course be applied to different flours as well as to two portions of the same flour.

Summary

In the multiple bleaching of flour with chlorine and hypochlorous acid either order of sequential application of these reagents has been

found to be greatly superior from the color removal standpoint to the simultaneous application of the two gases to the flour.

The multiple bleaching of flour with three gaseous reagents does not seem commercially feasible. It offers no distinct advantages either from the color removal or baking standpoints.

Very efficient bleaching of the color and great flexibility in effects on baking characteristics may be obtained by blending two of the same or different flour streams bleached with two different gaseous reagent multiple bleaching combinations.

Many possible bleaching treatments have been described. To recapitulate the findings in this and the preceding papers of this series, the greatest bleaching efficiency in the application of two gaseous reagents to flour results when the reagent higher up in the following list is applied first, followed by the one lower in the list:

Chlorine
Hypochlorous acid
Chlorine dioxide
Nitrogen trichloride
Nitrogen peroxide

No preference in order exists for the combination of chlorine and hypochlorous acid or that of nitrogen trichloride and hypochlorous acid.

Acknowledgments

The authors wish to acknowledge the assistance of B. A. McClellan in the baking evaluations of the experimentally bleached flours.

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FLOUR BLEACHING—EXPERIMENTAL APPARATUS AND PROCEDURES [1]

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The purpose of this paper is to describe the generating and metering equipment, techniques, and methods of analysis used by the writers in the handling of solid and gaseous reagents for flour bleaching experiments. Results obtained with this equipment and these procedures are given in the series of papers on multiple bleaching of flour by Ferrari *et al.* (1945 and 1947).

Flour Agitation

Laboratory Apparatus. A simple means of flour agitation is used for the laboratory batch-scale experiments. This apparatus consists of MacLellan batch mixers, preferably of stainless steel with hollow shafts to facilitate the application of the bleaching gases. The maximum capacity of each unit is about 8 pounds of flour. As little as 2 pounds of flour can be bleached, but it is not advisable to go below 2 pounds. Accuracy increases as the amount of flour bleached increases, so a 4- or 8-pound sample is better than a 2-pound sample. Since these mixers rotate at a slow speed of about 5 rpm, the proportion of flour suspended in the air at any given instant is relatively low in comparison with that produced by high-speed commercial agitators. Consequently, the selected amount of gaseous reagent must be applied slowly in order to produce uniform and optimum results. The total bleaching time for a capacity batch was found to be 20 minutes per application. The MacLellan batch mixers contain a set of 6 well-designed baffles placed properly with respect to the design of the shell and the speed and direction of rotation to achieve excellent mixing action and constantly expose new flour surfaces. For the most accurate results, a mixer of this type with baffles is believed to be necessary.

The gas is conducted from the generator by means of a flexible tube and is admitted to the mixer through the hollow shaft by means of a special Bakelite connection (see Figure 2), the hollow shaft of the mixer being lined with a glass tube to prevent corrosion of the metal and subsequent flour contamination. The opposite shaft is hollow with an opening which is partially filled with glass wool or cotton to

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prevent pressure build-up. Perfectly uniform color removal throughout the batch is obtained with this type of agitation. The results are easily replicated.

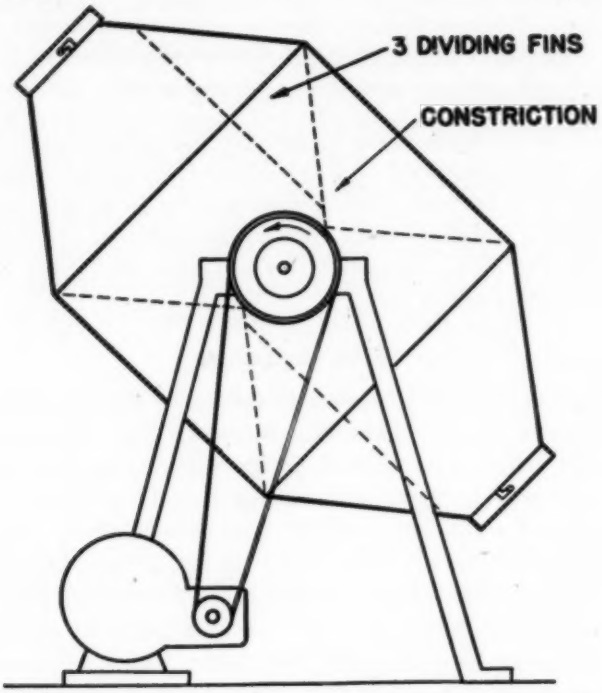

Fig. 1. ½ Batch bleaching agitator—MacLellan mixer with hollow shafts.

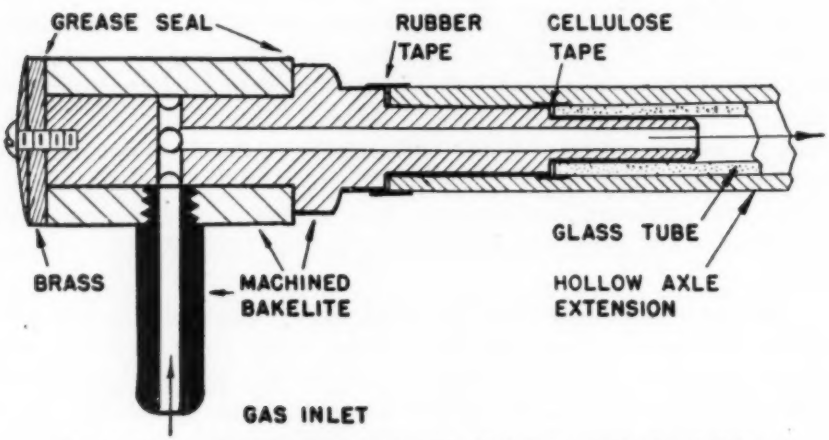

Fig. 2. Diagram showing construction features of bakelite connection for admitting gaseous bleaching reagents to laboratory batch mixer.

Larger mixers of this same type have been found effective in bleaching greater quantities of flour experimentally.

Pilot-Scale Equipment. The pilot-scale agitator consists of a chamber in which paddles rotate at high speed to simulate the action of a commercial agitator. This machine is contained in equipment capable of handling a continuous flour stream of 1 to 5 barrels per hour.

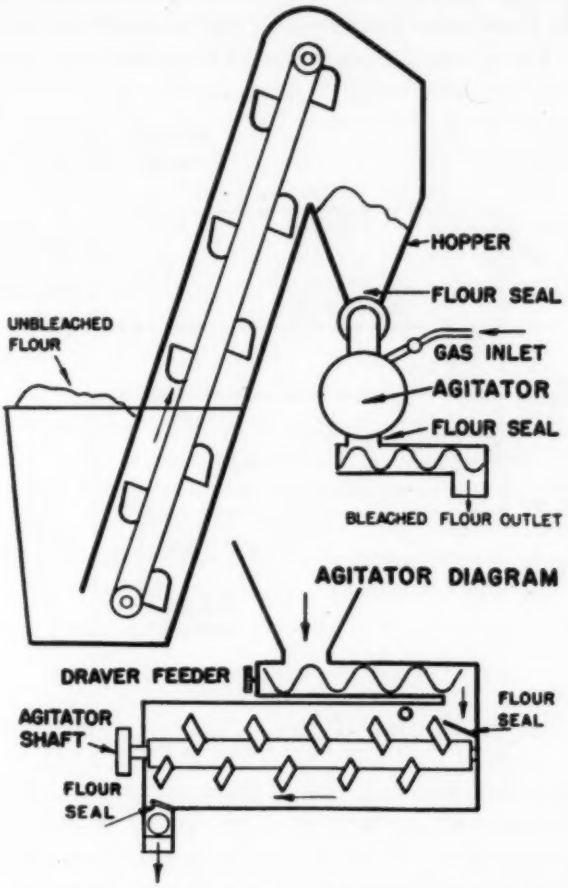

Fig. 3. Diagram of pilot-scale flour-handling and agitating equipment.

A Draver screw conveyor delivers a uniform flour stream to the agitator which has a flour seal at the flour inlet and exit points. Any desired bleaching gas or combination of gases can be admitted to the agitator chamber at a point directly below the flour inlet. Four gas inlets fitted with shut-off valves are provided to allow maximum flexibility. For multiple bleaching experiments with this apparatus, the flour must be recycled for each bleach which is to be applied separately or in sequence. Since the bleaching action is continuous, gases generated by commercial equipment as well as by experimental

apparatus may be used. The desired amount of gas is metered to the agitator and any excess is by-passed into an exhaust hood.

Commercial Agitators. Commercial agitators are essentially the same as the pilot equipment described except for their greater size and capacity. For multiple bleaching work, two or more agitators are connected in series with flour seals at the flour inlet and exit points of each unit. The gases are introduced at a point just below the flour inlets.

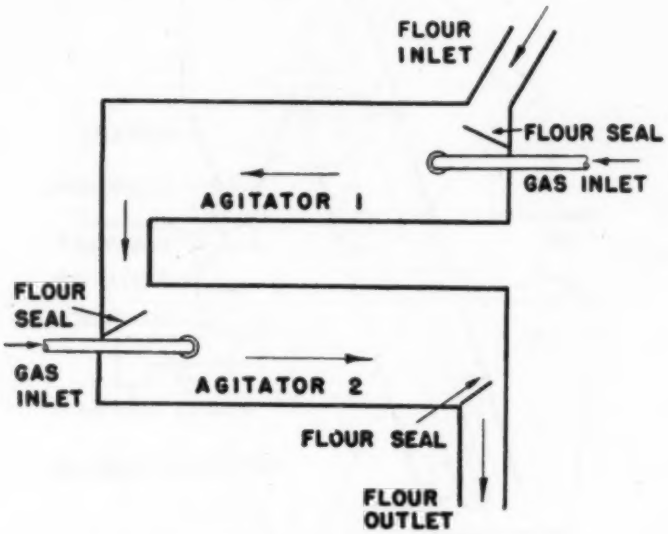

Fig. 4. Diagram of multiple bleaching agitator system.

Bleaching with Solid Reagents

Solid bleaching reagents, such as those containing benzoyl peroxide or fatty acid peroxides, are added before, during, or after the treatment with gaseous reagents. They are added as a single treatment in batch or pilot-scale experiments in MacLellan batch mixers of various sizes. At least 15 minutes is allowed for the blending of the reagent and the flour. From 10 to 36 hours is needed for the full bleaching effect of solid reagents to take place; so the color analysis is not made on the treated flour sample until sufficient time has elapsed.

Bleaching Gas Generators and Procedures

Apparatus utilized for the generation and metering of various bleaching gases provides for small-scale production of these gases with sufficiently flexible and accurate control to perform multiple bleaching and other bleaching experiments on flour. In a few instances indicated

below, available commercial equipment is used where feasible for batch and pilot-scale experiments.

Accurate control of each gas is accomplished by metering or weighing one constituent of the reaction mixture producing the gas. The gas is stripped from the reaction zone and conducted to the bleaching agitator by the use of metered air. The efficiencies of the reactions are determined by analysis of the effluent gases.

I. Chlorine Dioxide. Equipment or apparatus and procedures for the carefully controlled generation and metering of chlorine dioxide for flour bleaching on a laboratory, pilot, or commercial scale have been fully described in recent publications by Hutchinson and Derby (1945) and Woodward, Petroe, and Vincent (1944). In each case the method used is the gaseous chlorine, solid chlorite process of Hutchinson and Mecham (1943). Procedures for the analysis of the effluent gaseous mixtures for chlorine dioxide are given in the first two references cited above.

II. Nitrogen Trichloride. A. LABORATORY SCALE. Small-scale equipment for the generation of nitrogen trichloride consists of a gas-measuring burette and reaction chamber with proper connections for stripping the gas with metered air. When the gas is prepared in a reaction chamber and this unit amount of gas is stripped from the chamber and conducted to the flour, accurate metering of the air is not necessary for the bleaching process. However, with the equipment used, there was found to be an optimum rate of stripping air of about 300 ml per minute.

A diagram of the apparatus is shown in Figure 5. The procedure for generating nitrogen trichloride with this particular equipment is as follows: The chlorine line is filled with pure gas up to point *B* and the desired quantity is metered into the burette, displacing a saturated salt solution. The reaction tube which is first filled with a solution containing about 6 g of ammonium chloride per 200 ml of water is placed under vacuum sufficiently strong to draw all the metered chlorine into the tube through the porous diffusing cylinder. The vacuum is completely released by allowing air to enter the system at point *G* in order to flush the remaining chlorine into the reaction vessel. The reaction tube is then shaken to insure that all the chlorine is reacted, and the resulting nitrogen trichloride is removed from the tube by forcing the stripping air through the porous cylinder. The stripping time allowed for a 200 ml reaction tube was about 20 minutes, although most of the nitrogen trichloride is carried to the agitator in the first few minutes.

According to Ferrari (1928), nitrogen trichloride is produced according to the following over-all equation:

$$3\ Cl_2 + NH_4Cl = NCl_3 + 4\ HCl$$

The volume of chlorine needed for the bleaching treatment was calculated from this equation, proper corrections being made for pressure and temperature conditions. An excess of ammonium chlo-

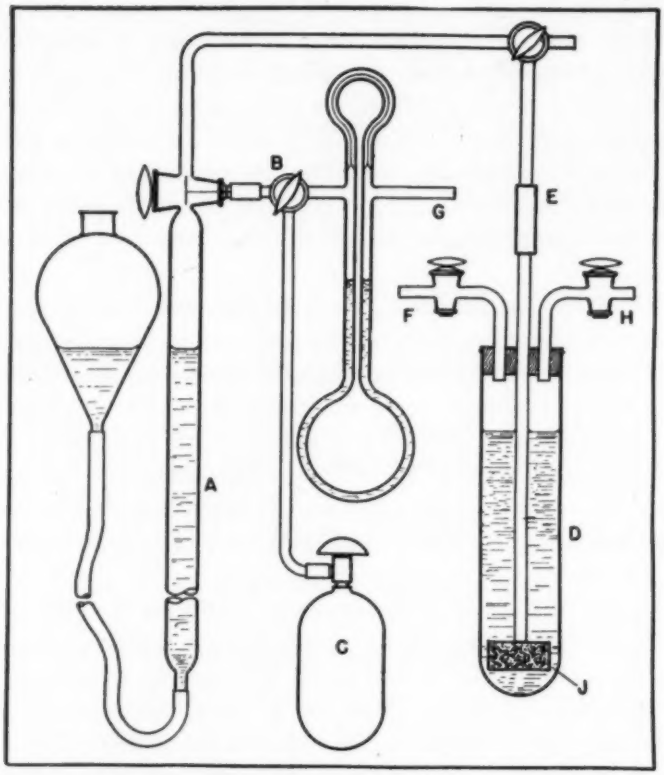

Fig. 5. Laboratory scale apparatus for the production of nitrogen trichloride. A. Gas burette. B. 3-way stopcock. C. Chlorine cylinder. D. Reaction tube. E. Flexible section. F. Vacuum connection. G. Air inlet. H. To agitator. J. Porous cylinder.

ride was always present, so the control of the treatment was on the chlorine input.

Bleaching results with this generator and the MacLellan batch mixer were found to be easily replicated, two samples of the same flour treated with the same dosage of NCl_3 usually varying less than 0.03 ppm in carotene content.

Ferrari, Hutchinson, and Mecham (1945) found that the efficiency of bleaching with this laboratory-scale generator and the MacLellan batch mixer is greater than in the nitrogen trichloride pilot-plant

equipment which in turn is more efficient than the commercial-scale equipment.

As a check on the proper functioning of the apparatus and application of the nitrogen trichloride gas to the flour, carotene analyses are usually made on the flour both before and after the bleaching treatment. Through experience in bleaching certain grades of flour, this provides a good check on the probable accuracy of the treatment; much better than can be obtained by the "Slick" or Pekar test. The carotene value is, of course, valuable for other reasons.

Flour color is expressed as "carotene" and is measured by the procedure described by Binnington, Hutchinson, and Ferrari (1941). Briefly stated, flour pigments are extracted with water-saturated butyl alcohol and the extract read in an Evelyn photoelectric colorimeter using a 440-mμ filter. The carotene results are converted to the more customary naphtha-alcohol carotene basis by using the following equation established by Binnington, Sibbitt, and Geddes (1938): Naphtha-alcohol "carotene" = 0.8075 (Butyl alcohol "carotene" − 0.14).

B. PILOT SCALE. Commercial-scale Agene equipment supplied by the Wallace and Tiernan Company is used with the pilot-scale conveyor and agitator equipment already described.

This commercial equipment may be used also for small-scale batch bleaching by maintaining a predetermined level of nitrogen trichloride generation and conducting a part of the gas through a flow meter into the flour blenders or agitators for a definite period of time. The treatment is calculated from the following factors: (1) the proportion of the total effluent diluted gas from the generator that is conducted to the flour, (2) the nitrogen trichloride level in the generated gas, (3) the time the gas is applied to the flour, and (4) the amount of flour treated. The excess gas is conducted into an exhaust hood or absorbed in caustic alkali or sodium sulfite.

III. Hypochlorous Acid. The following equation represents the probable course of the reaction that is utilized in the production of hypochlorous acid:

$$Ca(OCl)_2 + CO_2 + H_2O = CaCO_3 + 2\ HOCl \underset{air}{\uparrow}$$

Hypochlorous acid as a gas is used in quantities up to 25 g per barrel to bleach small unit amounts of flour (2 to 8 pounds) in the MacLellan blenders. This reagent may be generated according to the above equation by reacting a calculated amount of powdered calcium hypochlorite of known available chlorine content in a closed chamber with an excess of carbon dioxide in the presence of moisture, the hypochlorous acid being stripped from the reaction chamber by air.

Hypochlorous acid may be prepared by first weighing a small calculated amount of calcium hypochlorite of about 60–70% available chlorine content into a 1-liter Erlenmeyer flask. (For a dosage of 25 g hypochlorous acid per barrel on 4 pounds of flour, 1.23 g of calcium hypochlorite at 61.5% available chlorine content is required.) About a gram of moisture is added to the flask in the form of steam in such a

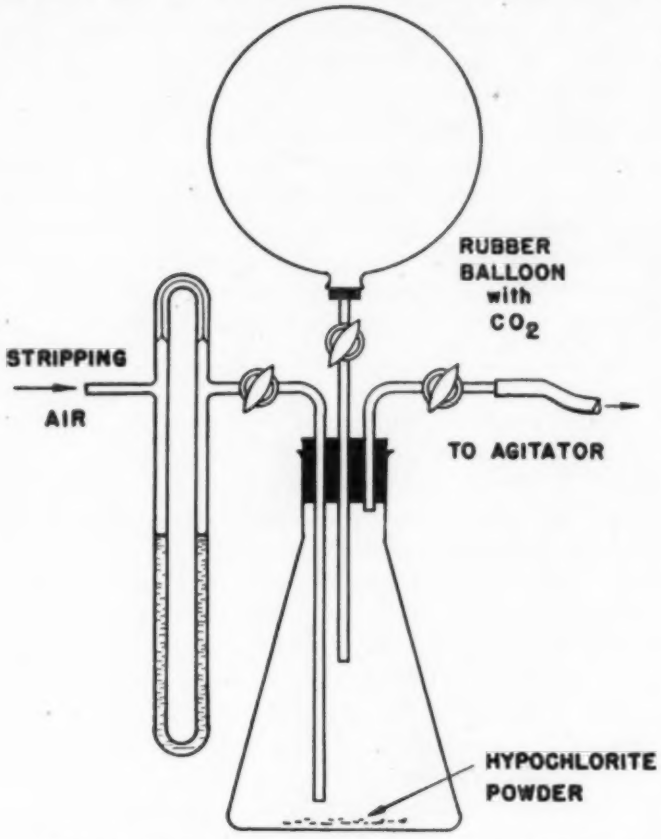

Fig. 6. Apparatus for the small-scale generation of hypochlorous acid.

manner that no large droplets form at any one point in the flask. The stopper containing the stopcock and tubing is inserted immediately and the flask partially evacuated with a water pump. The vacuum is released with carbon dioxide from a balloon. At room temperatures this method has an optimum reaction time of about 1½ to 2 hours in the dark, efficiencies of 93 and 89%, respectively, having been found for these reaction times. Optimum yield of hypochlorous acid depends both on the time of reaction and the amount of moisture present in the flask. The amount of moisture found necessary is that which

will be sufficient for the needs of the reaction in contact with the calcium hypochlorite as liquid or vapor and which is present in small enough excess so that the excess will be completely evaporated by an air flow of about 300 ml per minute for 20 minutes, which is the amount of air used for stripping the hypochlorous acid from the reaction vessel. If more moisture were present, the stripping time would have to be increased until the flask is dry; otherwise, some of the hypochlorous acid remains dissolved in the moisture in the flask. If too little moisture is present, the reaction does not reach completion, and the percentage yield will drop off considerably.

ANALYTICAL DATA: The analysis of the bleaching powder is determined by titrating with standard thiosulfate the iodine liberated from potassium iodide added to a dilute solution of the calcium hypochlorite acidified with dilute sulfuric acid.

The percentage yield of hypochlorous acid in the process described is determined in two steps. First, hypochlorous acid is generated in a flask as described, and after allowing 1½ hours to complete the reaction, the flask is swept with air at 300 ml per minute for about 20 minutes, at which time no noticeable moisture remains. The residue is then analyzed by the method indicated above for the bleaching powder. It has been found that over 99% of the available chlorine reacts and is displaced from the flask. Thus it becomes possible to prepare hypochlorous acid in a flask as described and conduct an analysis of its oxidizing power or yield by adding 50 ml of 1 *N* sodium hydroxide solution to the flask by means of a pipette and a rubber connector and shaking the flask in order to dissolve or react all of the hypochlorous acid. Since the reaction to produce the hypochlorous acid creates a slight vacuum in the flask, the sodium hydroxide solution can be readily admitted.

After allowing a short time for the sodium hydroxide to react with the hypochlorous acid, potassium iodide is added to the solution, after which it is neutralized to a phenolphthalein end point and made acid with 10 ml of glacial acetic acid. From a standard thio titration, the amount of hypochlorous acid produced can be calculated.

IV. Nitrogen Dioxide. In order to conduct laboratory flour-bleaching experiments with nitrogen dioxide gas, a commercial Alsop generator complete with standard electrical equipment and blower for supplying a flow of air to the flaming arc dome is assembled according to the diagram in Figure 7.

A 5-gallon metal container equipped with inlet and outlet pipes is used in the air line following the Roots Connersville blower as an expansion chamber in order that the blower pulsations will not be transmitted to the flowmeters and arc discharge unit. The total air

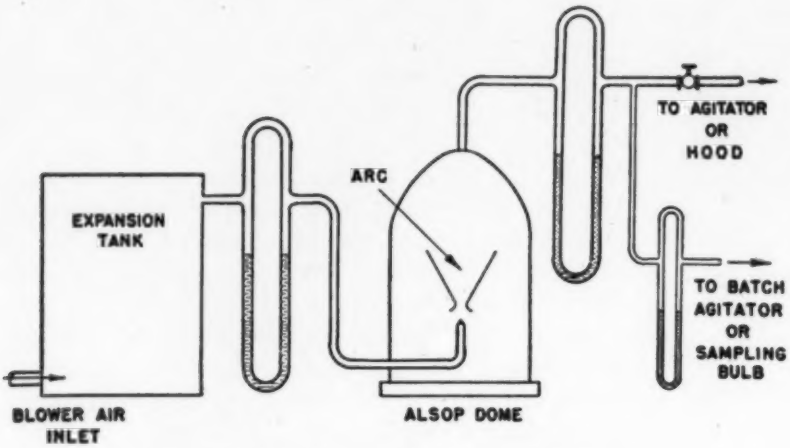

Fig. 7. Diagram of Alsop nitrogen dioxide generator with accessories for laboratory bleaching experiments.

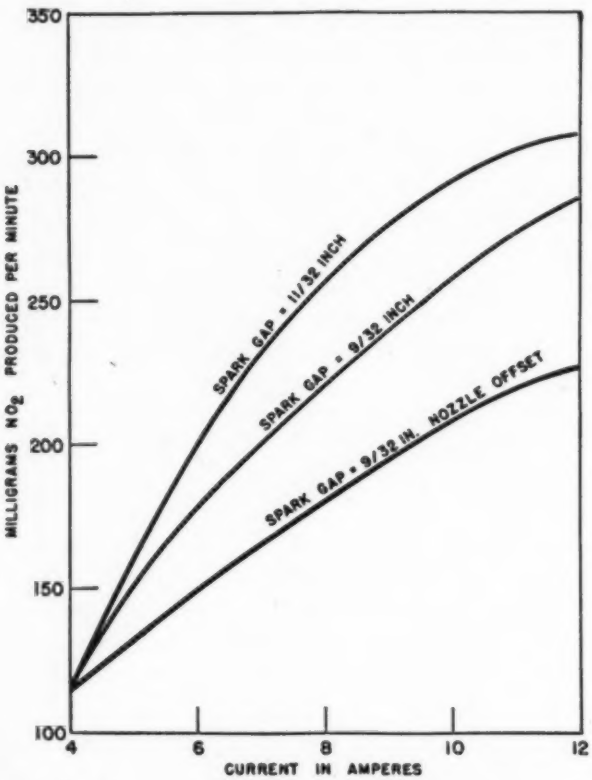

Fig. 8. Performance of test Alsop machine with constant air flow at 0.36 liters per second and with various spark gap adjustments.

flow is measured by the differential flow meters on each side of the Alsop dome. As the dome is heated by the arc discharge, the air volume becomes greater at the dome and the downstream flowmeter indicates a greater gas flow. However, the length of the system with subsequent cooling of the gas tends to cancel this effect, and it is not considered in the analytical calculations.

Before any bleaching work was done with the Alsop apparatus, it was thought desirable to calibrate the output of the machine. The

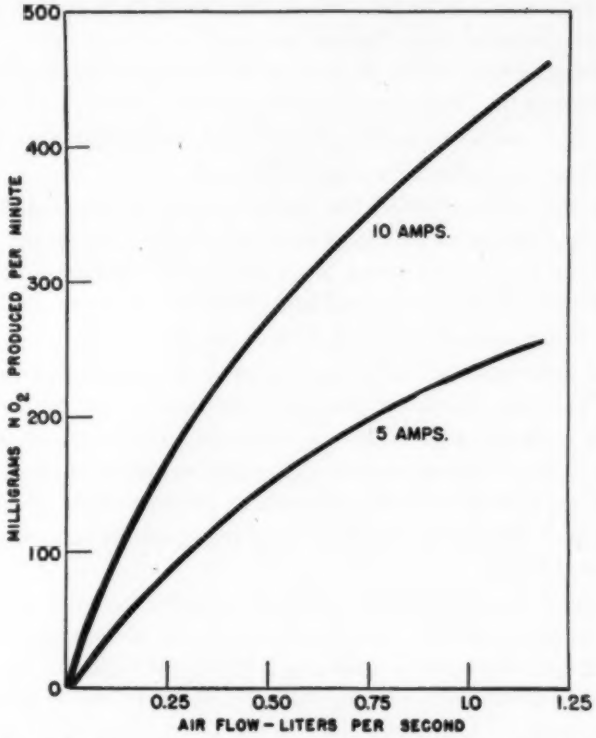

Fig. 9. Effect of air flow and amperage on the nitrogen dioxide production of the Alsop machine.

effluent gas was analyzed while the generator was operating at various measured air flows and at various levels of current input to the arc. The electrode spacing was also varied, as was the position of the electrodes over the air nozzle.

During the preliminary calibration, it soon became apparent that although the amount of nitrogen dioxide produced is controlled primarily by air flow and amperage, the other factors also have an important effect on the nitrogen dioxide output. The nitrogen dioxide production, of course, increases with current. Analysis of the effluent gases showed the extent to which the change in the spacing between the

electrodes affects the nitrogen dioxide production. Also demonstrated was the fact that nitrogen dioxide production was lowered considerably if the electrodes were not in optimum alignment with the air nozzle, an adjustment that appears to be very critical.

The nitrogen dioxide productions with constant air flow for electrode gap settings at 11/32 inch and 9/32 inch and also for the gap at 9/32 inch with the electrodes offset slightly from the air nozzle are shown in Figure 8. It is seen that the nitrogen oxide output increases with increased space between the electrodes. It is greatest when the spark gap is centered over the air nozzle.

The effects of variations in air flow on nitrogen peroxide output are shown in Figure 9. For each amperage level investigated the production of nitrogen oxides is increased markedly as the air flow is increased over the range permitted by the equipment.

During the calibration of the Alsop generator, the main gas line is exhausted to a hood, and a small amount of gas (300 ml per minute) is diverted into a gas sampling bulb of known volume (Figure 10). Because of partial pressure considerations, the total air flow is held at about 0.36 liters per second or 21.6 liters per minute as measured by the differential flow meters. The gas valve in Figure 7 has to be closed somewhat in order to divert the required flow of gas to the sampling bulb. The back pressure caused by this action is measurable, although very slight. The flowmeters are calibrated under practically the same back pressure; however, such refinements are not considered necessary and in terms of bleaching effect usually represent an insignificant flour color removal value.

When the Alsop machine is considered to have reached equilibrium using any desired setting from 4 to 12 amperes of current to the transformer, the bulb sample is taken. The 50 ml bulb on the sampling device is blown out with air and filled with 50 ml of 1 *N* sodium hydroxide solution. A stopper is placed in the small bulb, and the sodium hydroxide solution is allowed to flow down into the sample chamber by opening the connecting stopcock and adjusting the three-way stopcock in such a manner that the gas may circulate throughout the whole closed apparatus. The absorption or reaction time allowed varies from 3 hours to overnight. Analytical procedure: the reaction which takes place in the sampling bulb between nitrogen dioxide and sodium hydroxide may be shown by the equation:

$$2\ NO_2 + 2\ NaOH \rightarrow NaNO_2 + NaNO_3 + H_2O$$

Thus the nitrite formed represents one-half of the nitrogen dioxide in the sampling bulb. The nitrite formed is determined quantitatively

by the colorimetric method of Griess and Ilsovay, as described by the Association of Official Agricultural Chemists (1935).

The standard nitrite solution is prepared by dissolving 0.1097 g silver nitrite in 20 ml of hot water, precipitating the silver with 0.10 g of sodium chloride, and making up to 1 liter. When 10.00 ml of this

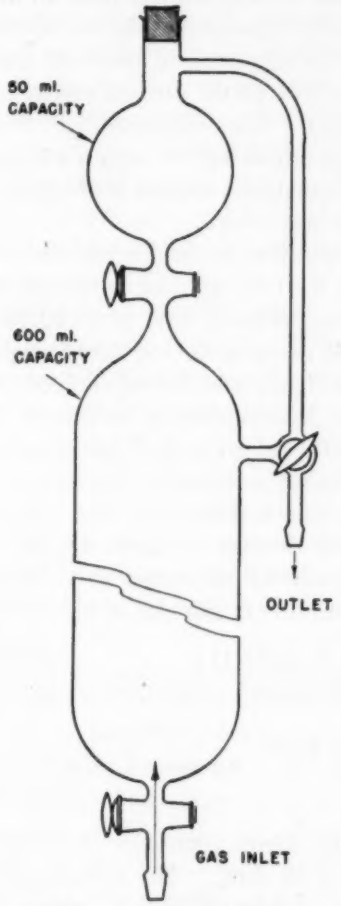

Fig. 10. Gas sampling bulb.

solution is diluted to 1000 ml, a standard solution is obtained which contains 3.28×10^{-4} mg of nitrite ion per ml. The solutions of sulfanilic acid and α-naphthylamine hydrochloride are prepared as directed in the above citation. The reference color standards are made up daily containing from 3.00 ml to 6.00 ml of standard nitrite solution by 0.25 ml increments diluted to 100 ml in matched Nessler tubes. Small differences in nitrite concentration are most easily distinguished in this range.

Since the gas sample is absorbed in alkali, the effect of the addition of alkali to the color standards was studied in connection with the rate of color development and the final color obtained. The effect of the sodium chloride produced by neutralizing the sodium hydroxide with hydrochloric acid was also determined.

The results of these tests indicated that an addition of more than 0.5 milliequivalents of sodium hydroxide had a definite retarding effect on the rate of color development and the final color obtained. It was also noted that this effect could not be controlled by neutralization with hydrochloric acid. The dilutions of the absorbed nitrogen dioxide solutions are manipulated in such a way as to insure that only 0.025 to 0.10 milliequivalents of sodium hydroxide are present when the color-forming reagents are added.

After the gas sample has reacted with the 50 ml of 1 *N* sodium hydroxide solution, a 5.00 ml aliquot is withdrawn and diluted to 1 liter. From this diluted aliquot, 5.00 ml to 20.00 ml, according to the strength of the sample, are transferred for the colorimetric determination to a 100 ml Nessler tube and 2.0 ml each of the sulfanilic acid and the α-naphthylamine hydrochloride solutions are added and the volume made up to 100 ml with distilled water. After mixing and standing at least one hour, stoppered, the Nessler tube containing the diluted sample which has achieved a color proportional to the concentration of the nitrite therein is matched with one of the tubes containing the standard nitrite solution, and the amount of nitrogen dioxide produced per minute is calculated by the following equation:

$$\text{Mg } NO_2/\text{min} = \frac{(\text{ml N NaOH})}{\text{ml aliquot}} \frac{(\quad \text{ml dilution} \quad)}{\text{ml diluted sample to Nessler tube}}$$

$$(\text{ml standard})\ (3.28 \times 10^{-4}) \frac{(\quad 1000 \text{ ml} \quad)}{\text{ml vol. of sample}}$$

$$(\text{air flow, liters/min}) \quad (2)$$

Sample calculation: the Alsop generator is operated at 12 amps. and 21.6 liters per minute of air flow. The volume of the sample is 618 ml. After reacting the gas with 50 ml of 1 *N* sodium hydroxide solution, a 5.0 ml aliquot is diluted to 1 liter. From this diluted solution a 4 ml aliquot in the Nessler tube with the color-forming reagents and water matches the tube containing 5.25 ml of the standard solution. The milligrams of nitrogen dioxide produced per minute in the effluent gas is calculated by substituting in the above equation:

$$\text{Mg } NO_2/\text{min} = \frac{50}{5} \times \frac{1000}{4} \times 5.25 \times 3.28 \times 10^{-4} \times \frac{1000}{618} \times 21.6 \times 2 = 300$$

The various sample dilutions may be manipulated to suit particular requirements with the minimum dilution dependent on the concentration of alkali in contact with the color-forming reagents as previously mentioned.

Batch-Scale Bleaching with Nitrogen Peroxide

After the generator is calibrated for one air flow and a range of ampere settings, it can be used for accurate small-scale batch bleaching. The assembly is practically the same as for the calibration, with most of the gas being exhausted into a hood. A measured or metered part of the total known flow of gas is admitted to the small MacLellan mixer in which a weighed amount of flour is being agitated. This metered amount of gas is introduced to the flour for the length of time shown by the calibration data to be required to produce the desired treatment of the flour.

Convenient time and flow for small dosages may be worked out at 10 amperes of current so that the generator is working near capacity. Then, in the event large-scale bleaching is desired, the apparatus need not be changed, the dosage being regulated by the amount of gas introduced to the flour.

V. Chlorine. A. Laboratory Scale. The test bleaching of flour with chlorine gas is easily and accurately accomplished by use of the laboratory nitrogen trichloride apparatus previously described. The apparatus is modified to the extent of exchanging the reaction tube for a 250 ml wide-mouth bottle fitted with two long inlet tubes and one short exit tube as shown in Figure 11. This bottle *D* acts as a gas-mixing chamber which allows metered chlorine from *A* through *E* to be diluted with air conducted from *G* through *F* (three-way stopcock *B* being closed), which sweeps it to the blender and flour.

Considering the nature of the flour agitation in a MacLellan blender, it is recommended that the chlorine be admitted to the gas and air mixing chamber at a rate not to exceed 25 ml per minute with the air flow at about 300 ml per minute. This procedure will produce a uniform bleach throughout the sample. Uniformity of results is not so easily obtained if the rate of chlorine application is too great.

After all of the chlorine is forced from meter *A* by the leveling bulb, line *E* is opened to the inlet air by partially closing pinch clamp *F* and opening stopcock *B* to this air flow. This operation insures that all of the metered chlorine in the apparatus is conducted to the flour.

Since no reaction takes place, no analytical control is necessary for this apparatus.

B. Pilot Scale. The pilot-scale bleaching of a flour stream with chlorine is done with commercial scale equipment which is adjustable

for continuous flow on small streams. For a very small continuous gas flow, the output from the commercial apparatus may be proportioned by use of differential manometers. A metered part of the total known flow of gas may be admitted to the flour agitator.

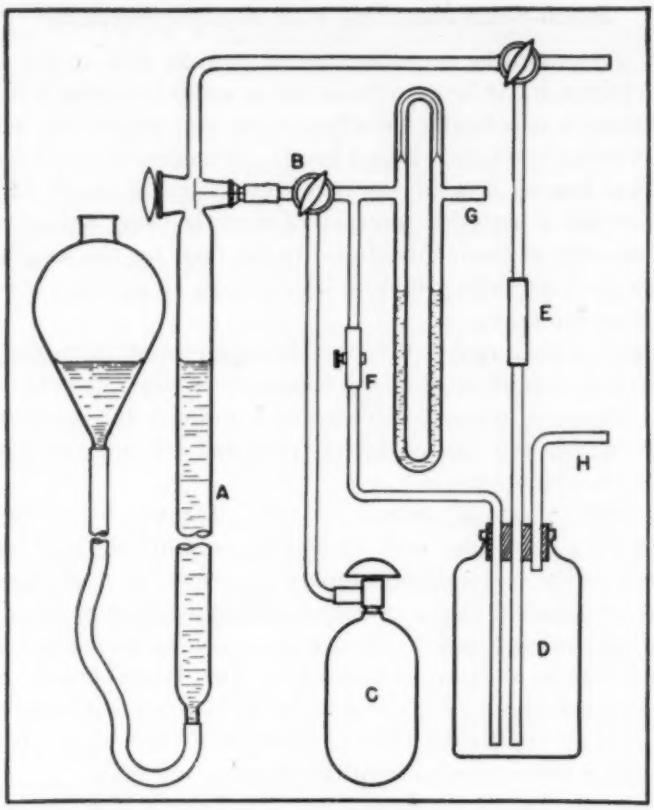

Fig. 11. Chlorine metering device for small-scale bleaching. A. Gas burette. B. 3-way stopcock. C. Chlorine cylinder. D. Gas mixing chamber. E. Flexible connection. F. Screw clamp. G. Air inlet. H. Gas outlet to flour agitator.

Summary

Apparatus and procedures for the experimental bleaching of flour with nitrogen trichloride, hypochlorous acid, nitrogen dioxide, and chlorine are described in detail and those using chlorine dioxide outlined briefly.

Agitating equipment suitable for flour bleaching on a laboratory, pilot, or commercial scale is described. The generation and metering of nitrogen trichloride, chlorine, and nitrogen dioxide for laboratory, pilot, and commercial practice are discussed. A method is described for the laboratory-scale production of hypochlorous acid.

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SOME FACTORS INFLUENCING THE PROTEIN, CYSTINE, AND METHIONINE CONTENT OF DRY PEAS [1]

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Cystine and methionine have been shown to be the limiting amino acids of the proteins of dry peas for nutrition (Johns and Finks, 1921; Woods, Beeson, and Bolin, 1943; Peterson, Lampman, Bolin, and Stamberg, 1944). Considerable variation in both the cystine and methionine contents of dry peas was reported by Evans (1945) for samples of peas obtained on the open market and differing in variety. They were probably grown in different years and under different climatic conditions.

Different varieties of soybeans have different nitrogen and cystine contents (Hamilton and Nakamura, 1940). Different varieties of wheats and wheats receiving different treatments have been shown to differ in sulfur and nitrogen (Greaves and Bracken, 1937), and in cystine content (Gubler and Greaves, 1942). The sulfur content of alfalfa was shown by Evans and Greaves (1937) to vary with variety, crop, and fertility of the soil.

It appeared desirable to study the influence of certain factors on the protein, cystine, and methionine in dry peas to see if the protein and sulfur of dry peas can be raised by some method of management or by use of particular varieties.

Materials and Methods

Alaska peas were used to study the influence of fertilization on the protein, cystine, and methionine in dry peas. The experimental plots were located in the Palouse area of eastern Washington. Six fertilizer treatments were used: sodium nitrate (100 pounds per acre), ammonium sulfate (100 pounds per acre), sulfur (20 pounds per acre), gypsum at the rate of 100 pounds per acre, gypsum at the rate of 200

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pounds per acre, and sodium nitrate and gypsum (each 100 pounds per acre). Each treatment was grown in each of three locations. One series was grown on the north slope, one on the south slope, and one on the hilltop.

The two varieties of peas produced in the Palouse area which are most used for dry peas are Alaska, and First and Best. These two varieties along with White Canada, a late-maturing pea, were studied to see if variety had any influence on the protein, cystine, and methionine content of dry peas. Each variety was replicated four times.

Alaska peas were used to determine the influence of date of harvest, or maturity, on the protein, cystine, and methionine in dry peas. Six plots were used, all planted at the same time. Duplicate plots were harvested on July 17, 1944, when the vines were beginning to show signs of maturity by the presence of some dead leaves although plants and pods were still definitely green. Two plots were harvested on July 22, 1944, when the vines were thought to be intermediate in maturity between the July 17 harvest and the mature stage. The remaining two plots were harvested on August 2, 1944, when the vines were completely dry. The peas were mature about July 29.

The samples were ground in a Wiley mill and well mixed soon after harvesting. The ground samples were than placed in bottles and left sealed till analyzed. All analyses were completed within a year after harvesting. Protein was determined by the Kjeldahl-Gunning-Arnold Method (A.O.A.C., 1945). Total sulfur was determined as described by Evans and St. John (1944), organic sulfur as described by Evans and Greaves (1937), and cystine and methionine by the differential oxidation procedure (Evans, 1945). The question of the specificity of this procedure for cystine and methionine might be raised when used with some materials containing other sulfur compounds than cystine, cysteine, methionine, and sulfate. Evans (1945) showed a close agreement between the differential oxidation procedure for methionine in soybean oil meal and cottonseed meal and the procedure of McCarthy and Sullivan (1941) when corrections were made for losses during acid hydrolysis. A similar study with six samples of dry peas gave an average value of 1.07% methionine in the pea protein by the McCarthy and Sullivan (1941) method and one of 1.03% methionine by the differential oxidation procedure. Differences between the two methods for individual samples were less than differences between different determinations on the same samples by the McCarthy and Sullivan procedure. No comparison could be made between the differential oxidation procedure for cystine and colorimetric methods, because no good method is available for hydrolyzing peas for cystine determination, owing to the large loss of cystine on hydrolysis of sub-

stances such as dry peas (Lugg, 1933). Isolation of the whole protein involves use of alkaline solutions which cause destruction of cystine. Because of this the differential oxidation procedure offers the best method available for the determination of cystine in dry peas.

The results were analyzed statistically by the method of variance (Snedecor, 1937). The variance for error represents variation between replicate plots, and not variation in chemical determinations. All chemical determinations were made in duplicate and the average values used.

Results and Discussion

Fertilizers. The addition of sulfur-containing fertilizers to the soil increased the percent of total sulfur in the dry peas grown thereon, but decreased the protein in these peas (Table I). This held true when

TABLE I

INFLUENCE OF FERTILIZER TREATMENTS ON PROTEIN CONTENT AND SULFUR DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*) [1]

Treatment	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methionine
	lbs/acre	%	%	%	%	%	%
Check (East)	1540	23.8	0.17	0.14	0.57	1.3	1.0
Sodium nitrate	1470	23.6	0.17	0.13	0.54	1.3	0.9
Sodium nitrate and gypsum	1550	22.9	0.21	0.14	0.58	1.6	0.7
Gypsum (low)	1500	22.1	0.22	0.14	0.63	1.8	0.7
Gypsum (high)	1450	21.1	0.22	0.14	0.65	1.8	0.8
Ammonium sulfate	1420	21.9	0.22	0.14	0.63	1.7	0.8
Sulfur	1600	23.5	0.20	0.14	0.56	1.5	0.8
Check (center)	1560	23.0	0.17	0.13	0.55	1.5	0.7
L. S. D.[2]		1.0	0.02	0.01	0.05	0.2	0.3
F value [3]		8.49	16.26	3.50	5.57	14.00	9.76

[1] These values are averages for the three locations. The peas were harvested when mature.
[2] L. S. D. indicates the least difference required for significance at the 5% level.
[3] F values required for significance at the 5% and 1% levels are 2.80 and 4.40.

sodium nitrate was applied with gypsum and when ammonium sulfate was applied, as well as when gypsum was used alone. There were practically no differences in organic sulfur content of the whole peas, but the protein of the peas fertilized with ammonium sulfate or gypsum alone contained significantly more sulfur than did the protein of the others. Cystine values in the protein were influenced in the same direction as the sulfur, but the methionine values were not significantly different. None of the differences found were large, and although certain of them were significant statistically, the importance of some of the differences from a nutritional standpoint is questionable.

That climatic conditions may have a significant influence on the composition of dry peas is evident from Table II which presents the average values for dry Alaska peas grown on the south slope, the north slope, and on the hilltop of one of the Palouse hills. Such slopes are common in the hilly Palouse country. The peas grown on the south

TABLE II

INFLUENCE OF LOCATION ON THE HILL ON PROTEIN CONTENT AND SULFUR DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*) [1]

Location	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methionine
	lbs/acre	%	%	%	%	%	%
South slope	1580	23.9	0.22	0.14	0.59	1.5	0.9
Hilltop	1160	21.6	0.19	0.13	0.59	1.6	0.7
North slope	1450	22.8	0.19	0.14	0.59	1.6	0.8
L. S. D.	—	0.6	0.01	0.01	0.03	0.1	0.2
F value [2]	—	34.03	17.29	19.25	0.32	2.92	2.39

[1] Eight plots each receiving a different fertilization treatment were harvested from each location. The peas were harvested when mature.
[2] F values required for significance at the 5% and 1% levels are 3.74 and 6.51.

slope were richer in protein and sulfur than those grown on the north slope or the hilltop, although the differences were quite small and their importance from a nutritive point of view might be of little importance. No significant differences in the cystine, methionine, or sulfur in the pea proteins were observed.

TABLE III

INFLUENCE OF HARVESTING DATES ON PROTEIN CONTENT AND SULFUR DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*) [1]

Harvesting date	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methionine
	lbs/acre	%	%	%	%	%	%
July 17, 1944	1110	26.4	0.23	0.15	0.58	1.3	1.1
July 22, 1944	1338	26.1	0.23	0.15	0.58	1.2	1.2
Aug. 2, 1944	1254	25.8	0.24	0.16	0.61	1.3	1.3
L. S. D.	—	0.9	0.02	0.01	0.01	0.1	0.3
F value [2]	—	2.14	1.50	—	42.90	3.00	1.38

[1] Average values for two quadrates. No fertilizer was applied, but this field had been previously in alfalfa which was plowed under in 1943. The peas in this field were considered to be mature July 29, 1944.
[2] F values required for significance at the 5% and 1% levels are 9.55 and 30.81.

Maturity. Although the protein from the peas harvested at the latest date, August 2, appeared to contain more sulfur than peas harvested earlier, the differences were small and no other statistically

significant differences were observed (Table III). None of the differences would appear to be of sufficient magnitude to be important from a nutritive standpoint.

Variety. White Canada peas contained higher levels of protein, total and organic sulfur than did Alaska, or First and Best peas (Table IV). However, the proteins of these three varieties appeared

TABLE IV

INFLUENCE OF VARIETY ON PROTEIN CONTENT AND SULFUR DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*) [1]

Variety	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methionine
	lbs/acre	%	%	%	%	%	%
Alaska	726	22.1	0.20	0.16	0.70	1.9	1.0
First and Best	930	22.1	0.21	0.17	0.75	2.0	1.0
White Canada	648	23.6	0.22	0.18	0.74	2.0	1.0
L. S. D.	—	1.0	0.01	0.01	0.03	0.1	0.1
F value [2]	—	8.81	57.00	24.00	6.97	3.75	0.00

[1] Average values for four replications. No fertilizer was applied. The peas were harvested as soon as they were considered to be ripe.
[2] F values required for significance at 5% and 1% levels are 5.14 and 10.92.

to be similar in cystine and methionine contents. It appears from these results that the differences in cystine and methionine levels in the protein of dry peas reported by Evans (1945) were not due to differences in variety, but to some other factor. Further work with additional varieties of peas might show differences comparable to those reported for organic sulfur in wheat (Greaves and Bracken, 1937), cystine in wheat (Csonka, 1937; Gubler and Greaves, 1942), cystine in corn (Doty, Bergdoll, Nash, and Brunson, 1946), and cystine in soybeans (Hamilton and Nakamura, 1940).

Evans (1945) determined the percentage of cystine and methionine in five dry pea samples. Cystine values ranged from 1.4 to 0.9% of the protein and methionine values ranged from 1.6 to 0.8%. Similar variations are reported in the present paper with cystine values of 2.0 to 1.2% of the protein and methionine values of 1.3 to 0.7%. Taken together, cystine values from 2.0 to 0.9% and methionine values between 1.6 and 0.7% were obtained. Although fertilization with gypsum increased the cystine from 1.3 to 1.8% of the protein, no fertilization or soil management factors were observed which would explain the wide differences observed between the different samples referred to above. These must be left for further investigation, as must the reason why the ratio between cystine and methionine varied between 1:1 and 2:1.

Also of interest are the differences in protein content, unexplained by the data presented, of some of the samples of peas. The observation of Wakeham (1943) that crops grown under "poor" conditions may contain more nitrogen and more minerals than crops grown under "good" conditions may be of importance in this regard. Yield data presented for the present experiment do not indicate that yield and composition were in any way related.

Summary

A study was made of the influence of fertilizer treatment, climatic condition, stage of maturity, and variety on the percentage of protein, sulfur, cystine, and methionine in dry peas. The addition of sulfur-containing fertilizers increased the total sulfur and the cystine level of the peas, but decreased the protein. The location on the hill (slope) on which the peas were grown significantly influenced the percent of protein in the peas, but not the cystine or methionine in the protein. Neither harvesting date nor variety influenced the cystine or methionine content of the dry pea proteins, but White Canada peas contained more protein than Alaska, or First and Best.

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BOOK REVIEW

Organic Chemistry. By Paul Karrer. Translated by A. J. Mee with translations of additions and revisions to latest German edition by M. F. Darken. xx + 953 pp. Elsevier Publishing Co., Inc., New York, N. Y. 1946. Price $7.50.

The book under review is the second English edition of the well-known excellent *Lehrbuch der Organischen Chemie* by Paul Karrer which has been widely used by students of organic chemistry since the publication of the first German edition in 1928. The present text is based on the eighth German edition, which appeared in 1942.

The aim of the book is to provide students with a medium-sized textbook surveying the ever-increasing facts and theories of organic chemistry. Special attention has been paid to the description of methods of synthesis and to the determination of structure of organic compounds. Particular emphasis has been placed upon naturally occurring substances and biological topics; and the problems of stereochemistry are discussed in an excellent fashion.

The arrangement of the book is based upon the historical division of organic chemistry into aliphatic, carbocyclic, and heterocyclic compounds. In each section the compounds are regarded as functions of the hydrocarbons and have been arranged, as far as possible, according to their functional groups. There is also a short division dealing with compounds of heavy hydrogen and heavy oxygen and an appendix consisting of 30 tables covering a variety of subjects. These include tables on the production of coal, mineral oil, sugar, and textiles; tables giving the number of structural isomerides of various organic compounds; dissociation constants of organic acids and bases; and other interesting information.

When comparison is made with the first English edition, no extensive modifications are found in the text of the new edition. Most of the changes consist of small additions of new material. In all, about one hundred changes or additions have been made. A large number of these occur in those portions of the book dealing with natural products and biological processes where new discoveries have necessitated additions or modifications. One important contrast between the present and earlier English edition is the placement of greater emphasis upon the electronic concepts of structure resulting in revisions in those portions of the book dealing with structures, reaction theories, etc. The section which deals with the composition and analysis of organic compounds is unchanged. It is felt that this discussion deserves some revision, at least to the extent of employing illustrations of more modern equipment.

Since the material has been skillfully selected and is presented in an interesting fashion, the book should be well received by students of organic chemistry. It is well written and the price is considerably lower than the first English edition.

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Merck & Co., Inc. now manufactures all the vitamins commercially available in pure form, with the exception of vitamins A and D.

Merck research has been directly responsible for many important contributions to the synthesis, development, and large-scale production of individual vitamin factors in pure form.

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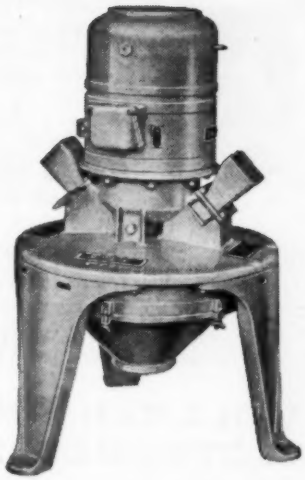

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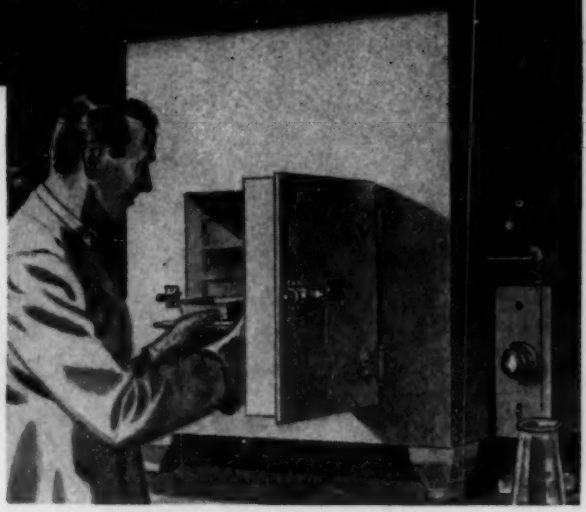

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